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Paediatric Schistosomiasis: Dynamics and Consequences

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Declaration

I declare that this thesis has been composed solely by myself, unless otherwise stated. Where others have contributed to elements of the work, this is clearly stated by acknowledgement in the text. This thesis has not been submitted in whole or in part for any other degree or professional qualification.

The data presented in this thesis forms part of a larger immuno-epidemiological study; "Childhood Schistosomiasis: a Novel Strategy Extending the Benefits/Reach of Antihelminthic Treatment", in collaboration with the University of Edinburgh and the University of Zimbabwe. I played a major role in the preparation and execution of field work to gather data and biological samples for further analysis. All other laboratory assays for this work, and the data analyses and interpretation are entirely my own work. Any contribution from colleagues and the field team, such as gathering participant data, sample collection and processing for storage, as well as parasitology diagnosis for large volumes of samples, are referenced in the text as appropriate. For the work described in Chapters 5 and 6, mass spectrometry analysis of serum samples was carried out by Human Metabolome Technologies (HMT; Yamagata, Japan), and metagenomic sequencing of stool DNA samples was carried out by Beijing Genomics Institute (BGI, Shenzhen, China); all other analyses are entirely my own work.

All publications included in this thesis are my own work and have been explicitly referenced in the text as appropriate. Copies of publications based on the work presented in Chapters 1, 3, and 6, are included in the appendix. Published work as part of my PhD training, related to, but do not form part of work presented in this thesis, are also included in the appendix.

Derick Nii Mensah Osakunor

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Abstract

Urogenital schistosomiasis, caused by the parasite helminth *Schistosoma haematobium*, is one of the major parasitic diseases affecting millions of preschool-aged children (PSAC), i.e. aged 5 years and below, in sub-Saharan Africa. Diagnosis is via microscopic detection of parasite eggs in urine, and treatment is by administration of the antihelminthic drug of choice, praziquantel. Epidemiological studies show that PSAC are infected as early as a year old, with negative impacts on nutrition, growth, cognition, and overall health. Despite recommendations to treat PSAC with schistosomiasis, this age group is still excluded from treatment programmes for various reasons including: a) the lack of a child-friendly formulation of praziquantel, b) lack of a coherent strategy to access PSAC for screening and treatment, and c) lack of compelling evidence on infection, disease and treatment dynamics. Currently, the global infection and disease burden is not fully known in this age group, and longitudinal studies to describe the incidence of infection and morbidity, especially the early events that occur during the very first infection and treatment in PSAC are lacking. In addition, the mechanistic pathways of disease, treatment, and immunity are poorly understood in this age group. Operational difficulties including obtaining parasitology samples for diagnosis, failure to detect light infections, and inadequate knowledge about risk factors have also contributed to a lower research focus on PSAC, relative to school-aged children and adults.

To address these, I completed a series of studies, based on a larger longitudinal study on paediatric urogenital schistosomiasis (population age range: 6 months–5 years) conducted in the Shamva district of Zimbabwe. The aim was to determine the dynamics of infection, morbidity and treatment of the first *S. haematobium* infection, and to examine the impact of a regular screening and treatment strategy on (re)infections. I also determined the early host metabolic changes associated with the first *S. haematobium* infection as well as the impact of schistosome infection on the gut microbiome, and how these relate to disease progression, morbidity and overall health.

I determined that 92% of microhaematuria, 38% of stunting, and between 9%–34% of malnutrition (depending on what index is used) are attributable to *S. haematobium* infection in PSAC; schistosome-positive children were more likely to present with microhaematuria (25 times) and stunting (2 times), compared to uninfected children. I demonstrated the annual incidence of first schistosome infections (17.4%) and urinary morbidity (microhaematuria; 20.4%), with significant incidences recorded every quarter. I showed that within 3 months of the first infection, a significant amount of urinary morbidity, i.e. microhaematuria (61%) occurs, and is resolved 3 months post-praziquantel treatment.

In PSAC with no history of schistosome infection, regular quarterly screening and treatment of the first *S. haematobium* infection reduces the actual time at risk of infection in the population, and results in reduced rates of subsequent new infections. A single praziquantel treatment of schistosome infections (upon first infection) was associated with reduced reinfection rates and intensity a year later; an effect comparable to that observed post-treatment in chronically-infected children.

In young children experiencing their first schistosome infection, there are significant increases (≥2-fold) in serum metabolites primarily linked with energy (glycolysis, pentose phosphate pathway, starch, and galactose) and purine metabolism. The observed changes were commensurate with increasing infection intensity and were restored 3 months post-curative antihelminthic treatment. The affected metabolic pathways and its implications on the natural adaptive metabolic responses were consistent with parasite survival and development of schistosome morbidity in PSAC, including malnutrition, stunting and poor physical and cognitive performance.

Metagenomic analysis of the gut microbiota showed that the abundance of bacteria and fungi phyla from *Proteobacteria*, *Ascomycota*, and *Basidiomycota*, differed between schistosome-infected versus uninfected children. Specifically, infection was associated with increases in

Pseudomonas, *Stenotrophomonas*, *Derxia*, *Thalassospira*, *Aspergillus*, *Tricholoma*, and *Periglandula*, and a decrease in *Azospirillum*. I found evidence of 262 antimicrobial resistance genes, from 12 functional drug classes, but these showed no association with individual-specific data, including schistosome infection. This points to microbiome dysbiosis as an additional consequence of schistosome infection, with implications for morbidity, immunity, and overall health.

Taken together, the findings of this thesis show that early in the first *S. haematobium* infection, PSAC present with significant morbidity, and this resolves quickly with praziquantel treatment. A routine screen-and-treat strategy will optimise the chances of detecting and treating infections early, while reducing the risk of new and reinfections. The findings further highlight microbiome and metabolic alterations during schistosome infection, which may be relevant for disease pathogenesis. This thesis presents an integrative approach to schistosomiasis studies in PSAC, which contributes to evidence on infection/disease burden and dynamics, the applicability of currently available tools in the diagnosis, treatment and control of schistosomiasis, as well as the systemic impacts of infection on the host microbiome, metabolism, and overall health. It also adds to the repository of information, by providing a novel metagenomics and metabolomics dataset of PSAC from Zimbabwe. The findings reaffirm the need for early diagnosis and treatment of schistosome infections in PSAC to avert accumulative morbidity, and will inform stakeholders in providing new and appropriate interventions targeted at reducing schistosome-related pathology in young children.

Lay Summary

Millions of preschool-aged children (PSAC), i.e. aged 5 years and below, in sub-Saharan Africa suffer from a neglected tropical disease caused by helminth parasitic worms, known as schistosomiasis (commonly referred to as bilharzia or snail fever). In these young children, the disease has severe consequences including impacts on nutrition, growth, cognition, and overall health. This thesis focused on the species that causes urinary and genital disease, *Schistosoma haematobium*, which accounts for about two-thirds of all schistosomiasis cases in Africa. The disease can be diagnosed by microscopic detection of parasite eggs in urine, and treatment is by administration of the antihelminthic drug of choice, praziquantel. Despite current recommendations to treat PSAC with schistosomiasis, this age group is still excluded from treatment programmes for various reasons including: a) the lack of a child-friendly formulation of praziquantel, b) lack of a coherent strategy to access PSAC for screening and treatment, and c) lack of compelling evidence on infection, disease and treatment dynamics. Currently, the global infection and disease burden is not fully known in this age group. Long-term research to track the early events that occur during the very first schistosome infection and treatment, as well as the associated health impacts are lacking. In addition, the molecular and biochemical processes underlying disease, treatment, and protection from infection are poorly understood in this age group.

In this thesis, I worked to address these knowledge and research gaps, by following a natural time-course of schistosome infections in PSAC (age range 6 months–5 years) living in a schistosome-endemic area in Zimbabwe (Shamva district). The aim was to determine the dynamics of infection, morbidity and treatment of the first schistosome infection, and to assess the impact of a regular screening and treatment strategy on (re)infection. I also determined the early changes that occur with metabolism (the set of complex life-sustaining biochemical reactions in the body), during the first schistosome infection. The impact of schistosome

infection on the ecosystem of microbes that inhabit the human gut (microbiota) and perform vital roles in nutrition and health, was also determined.

The results showed that during the first schistosome infection, PSAC quickly develop clinical disease, as measured by urinary morbidity (blood in urine). Schistosome infection and morbidity can be detected early in PSAC, using parasitology (microscopic egg counts) and dipstick techniques (detection of blood in urine) within 3 months of first infection, and morbidity is resolved 3 months after curative treatment with praziquantel. Chronic growth/nutrition-related morbidity including malnutrition and stunting are attributable to schistosome infection, and these indices can help identify high-risk groups and measure the health impacts of infection and treatment in endemic areas. In PSAC with no history of schistosome infection, regular quarterly screening and treatment of the first infection, reduces the actual time at risk of infection in the population and reduces the rates of subsequent new infections. A single praziquantel treatment of schistosome infections (upon first infection) was associated with reduced reinfection rates and intensity a year later; an effect comparable to that observed post-treatment in children with longer-term infections.

In young children experiencing their first schistosome infection, there are significant alterations in energy and purine metabolism, consistent with development of schistosome morbidity in PSAC, including malnutrition, stunting and poor physical and cognitive performance. These changes correlated with increasing infection burden and were restored to almost pre-infection levels, 12 weeks after treatment. Compared to uninfected children, schistosome infection was associated with significant alterations in the abundance of specific gut microbiota populations, which may be relevant for disease progression and negative impacts on overall health.

Overall, this thesis presents an integrative approach to schistosomiasis studies in PSAC, which contributes to evidence on infection/disease burden and dynamics, the applicability of

currently available tools in the diagnosis, treatment and control of schistosomiasis, as well as an understanding into the factors underlying disease progression and overall health. The findings reaffirm the need for early diagnosis and treatment of schistosome infections in PSAC to avert accumulative morbidity. It will also inform stakeholders in providing new and appropriate interventions, targeted at reducing the consequences of schistosomiasis in young children.

Acknowledgements

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Finally, thanks be to God for seeing me through this amazing journey!

Abbreviations

3-PG	3-Phosphoglyceric acid
ADP	Adenosine diphosphate
AF	Attributable fraction
AMP	Adenosine monophosphate
AMR	Antimicrobial resistance
ANCOM	Analysis of composition of microbiomes
ANOVA	Analysis of variance
AOR	Adjusted odds ratio
APC	Antigen-presenting cells
ATP	Adenosine triphosphate
BAZ	Body Mass Index–for–age Z-score
BGI	Beijing Genomics Institute
CAA	Circulating anodic antigen
CCA	Circulating cathodic antigen
CE	Capillary electrophoresis
CE-ESI-MS	Capillary electrophoresis electrospray ionization mass spectrometry
CE-MS	Capillary electrophoresis mass spectrometry
CE-TOF-MS	Capillary electrophoresis time-of-flight mass spectrometry
CI	Confidence interval
CR	Cure rate
EPI	Expanded Program for Immunisation
ERR	Egg reduction rate
ESI-MS	Electrospray ionization-mass spectrometry
FC	Fold change
FDR	False discovery rate
FOB	Faecal occult blood

G6P	Glucose-6-phosphate
GABA	gamma-Aminobutyric acid
GLM	General linear model
GMP	Guanosine monophosphate
HAZ	Height–for–age Z-score
HMT	Human Metabolome Technologies
HPLC	High-performance liquid chromatography
IL	Interleukin
IMP	Inosine monophosphate
IQR	Interquartile range
KMA	K-mer alignment
MANOVA	Multivariate analysis of variance
MDA	Mass drug administration
MS	Mass spectrometry
MT	Migration time
MUAC	Mid-upper arm circumference
MUACZ	Mid-upper arm circumference Z-score
NCBI	National Center for Biotechnology Information
NTD	Neglected tropical disease
OPLSDA	Orthogonal Projections to Latent Structures Discriminant Analysis
OR	Odds ratio
OSC	Orthogonal Signal Correction
PC	Preventive chemotherapy
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
PLSDA	Partial Least Squares Discriminant Analysis

POC	Point-of-care
POC–CCA	Point-of-care circulating anodic antigen
POC–CCA	Point-of-care circulating cathodic antigen
POCUS	Point-of-care ultrasonography
PR	Prevalence ratio
PSAC	Preschool-aged children
PZQ	Praziquantel
SAC	School-aged children
SD	Standard deviation
SE	Standard error
STH	Soil-transmitted helminths
TGF-β	Transforming growth factor beta
Th1	T-helper type 1
Th2	T-helper type 2
TNF	Tumour necrosis factor
TOF-MS	Time-of-flight mass spectrometer
TPP	Target product profile
Treg	Regulatory T-cell
UACR	Urine albumin–creatinine ratio (UACR)
VIP	Variable importance in the projection
WASH	Water, sanitation and hygiene
WAZ	Weight–for–age Z-score
WHO	World Health Organisation
WHZ	Weight–for–height Z-score

Contents

List of figures

List of tables

Chapter 1 Introduction

Parts of this chapter have been published as a review article (Osakunor *et al.,* 2018b), and a copy of the publication is included in **Appendix E**.

1.1 Background

After performing an autopsy on infected patients in Egypt in 1851, the German pathologist Theodore Maximilian Bilharz was first to describe a parasitic disease in humans, which became known as bilharzia (Bilharz 1853). Although the terms bilharzia or snail fever are still commonly used, the most widely accepted scientific term for the disease today is schistosomiasis (Ross *et al.,* 2002, World Health Organization 2020b). Schistosomiasis is a water-borne tropical and subtropical disease that mostly affects communities with limited access to both safe water and adequate sanitation provision (Gryseels *et al.,* 2006, Colley *et al.,* 2014, World Health Organization 2020b). World estimates show that over 250 million people are infected (World Health Organization 2020b), with sub-Saharan Africa accounting for more than 90% of cases (Steinmann *et al.,* 2006, Colley *et al.,* 2014, World Health Organization 2020b). In endemic areas children carry most of the infection burden, with about 123 million infected children worldwide. Of this number, at least 25 million are preschool-aged children (PSAC), i.e. children who are 5 years old and below (WHO Expert Committee 2002, World Health Organization 2011). The health impacts of schistosomiasis thus start during the preschool age, and include protein wasting, malnutrition, poor growth and cognitive development, anaemia, and reduced physical activity during childhood (Freer *et al.,* 2018, McManus *et al.,* 2018). Later life consequences of schistosomiasis include chronic diarrhoea, poor sexual and reproductive health, susceptibility to coinfections, organ-related cancers, chronic or end-stage organ damage, and in some serious cases, death (Gelfand *et al.,* 1967, Lichtenberg 1975, Ishida and Hsieh 2018, McManus *et al.,* 2018, Tuffour *et al.,* 2018).

Diagnosis is typically by microscopic detection of eggs in urine or stool, depending on the species, and treatment is by administration of the antihelminthic drug of choice, praziquantel (PZQ) (McManus *et al.,* 2018, World Health Organization 2020b). In schistosome-endemic areas, infection rates and intensity are typically highest in populations aged between 6–20 years (Woolhouse 1998, Mitchell *et al.,* 2011). This is because exposure to schistosome infection is cumulative (Woolhouse *et al.,* 2000) and infection prevalence and intensity increase with age (Wami *et al.,* 2014), up until the teenage years when worm burden begins to decline due to natural worm death and onset of development of partial acquired immunity (McManus *et al.,* 2018). Hence, schistosome control programmes follow preventive chemotherapy (PC) by mass drug administration (MDA) and monitoring of infections in school-aged children (SAC; ≥6 years old) and adolescents (WHO Expert Committee 2002).

Epidemiological studies have shown evidence of schistosome infection in PSAC as early as six months old (Woolhouse *et al.,* 2000, Bosompem *et al.,* 2004, Odogwu *et al.,* 2006, Mutapi *et al.,* 2011, Kemal *et al.,* 2019, Mutsaka-Makuvaza *et al.,* 2019, Sacolo-Gwebu *et al.,* 2019). However, PSAC are excluded from treatment through MDA programmes (Stothard *et al.,* 2011, Stothard *et al.,* 2013). The World Health Organization (WHO) published a major recommendation in 2011 to include PSAC for treatment, based on evidence of schistosome infection and treatment in this age group (World Health Organization 2011). To date, the 29 schistosome-endemic countries in Africa that have implemented MDA programmes are yet to include PSAC (World Health Organization 2018). Moreover, where vaccine trials have been conducted in schistosome-endemic areas, these have included children aged from 6–11 years old (Riveau *et al.,* 2018, Tendler *et al.,* 2018). This creates a "treatment gap" and a health inequity (Stothard *et al.,* 2011, Stothard *et al.,* 2013), while PSAC remain significant reservoirs for infection transmission and thereby hamper elimination efforts (Lelo *et al.,* 2014, Njenga *et al.,* 2014).

Several reasons have been given for the exclusion of PSAC from schistosome treatment programmes, especially for those that started before the WHO recommendation. These include the unavailability of a child-friendly formulation of PZQ, lack of a consensus on safety and dosing of the current tablet form of the drug, and the absence of a coherent strategy to access and treat PSAC (Stothard *et al.,* 2013, Mutapi 2015a, Mduluza and Mutapi 2017). The historical perspective of a low risk of infection and a low disease burden (Sacko *et al.,* 2011), the assumption that PSAC cannot mount a protective immune response to schistosome infection, or that treatment efficacy depends on a mature immune system have also contributed to this treatment exclusion (Mutapi 2015a). Even if infected, the impact on the health of PSAC was unknown or considered negligible. In addition, operational difficulties including obtaining parasitology samples for diagnosis, failure of conventional diagnostics to detect early and light infections in PSAC, and the ease of accessing SAC in schools have biased research focus towards older children (Colley *et al.,* 2014). This has led to a poor understanding of schistosome exposure, infection, disease dynamics and treatment in PSAC.

Although concerted efforts have been made by providing scientific evidence to refute such misconceptions and barriers to schistosome treatment and research focus, there is still a need for improved research and knowledge, as well as adapting and refocusing current control and intervention strategies in PSAC (Tchuem Tchuenté *et al.,* 2017). To date, schistosomiasis studies in PSAC have focused on describing the prevalence of infection and morbidity in endemic areas, while longitudinal studies are lacking. For instance, studies to describe the incidence of infection and morbidity, especially the early events that occur during the very first infection and treatment in PSAC are lacking. Knowledge of screening and treatment times for primary infections, and follow-up studies to determine the impact of such strategies on reinfection are also limited. In addition, studies elucidating the host-parasite interactions at the molecular level, that contribute to parasite survival and disease development in PSAC are needed. If we are to strengthen schistosomiasis elimination programmes, addressing these

knowledge gaps is important in order to deliver sustainable control of schistosome infection and disease, and to inform development of specific interventions targeted at reducing morbidity.

Therefore, the work presented in this thesis aims to address the knowledge gaps outlined above, in relation to infection and disease dynamics, and the health impacts of infection and treatment in PSAC, focusing on *S. haematobium* species. It also adds to understanding the mechanistic pathways of disease and treatment in this age group, as it relates to the host metabolic system and the gut microbiome (the assembly of genomes of the diverse ecosystem of microbes associated with humans).

In this chapter, I summarise the current knowledge of schistosome infection, host–parasite interactions and disease dynamics, as well as treatment and immune mechanisms as pertains to PSAC. In relation to these areas, I identify important knowledge gaps in paediatric schistosomiasis practice, and how the work presented in subsequent chapters of this thesis (with a focus on *S. haematobium* species) contributes to closing these gaps. The rationale and specific aims of the work presented in each chapter as well as an outline of this thesis are also included.

1.2 Schistosome biology and clinical relevance

Schistosomiasis is caused by parasitic trematode worms of the genus *Schistosoma*. The most common species that infect humans are *S. haematobium,* which causes the urogenital form of the disease, and *S. mansoni* and *S. japonicum* which cause intestinal disease (Colley *et al.,* 2014). The species *S. guineensis, S. intercalatum* and *S. mekongi* although rarely reported in humans, also cause intestinal disease (Gryseels *et al.,* 2006).

Like other trematodes, *Schistosoma* species have a complex life cycle consisting of both free-living and parasitic forms. A schematic diagram of the complete life cycle and its clinical relevance as used in this thesis is shown in **Figure 1.1**. Schistosome eggs are released by the

definitive vertebrate host, either directly or carried by rains into freshwater via urine (*S. haematobium*) or faeces (*S. mansoni* and other intestinal forms). The eggs can stay viable for up to about seven days before they reach fresh water. The life cycle of schistosomes however require an asexual reproductive phase within an intermediate host snail – for *S. haematobium* this is *Bulinus* spp., for *S. mansoni, Biomphalaria* spp., and for *S. japonicum, Oncomelania* spp. (Steinmann *et al.,* 2006). Thus, the life cycle and transmission may be interrupted at this point with snail control interventions. Upon reaching fresh water, the eggs hatch and release miracidia that swim by ciliary movement (guided by light and chemical stimuli) to locate and penetrate a suitable freshwater snail host. In the snail, miracidia multiply asexually into multicellular sporocysts and again into cercarial larvae (the free-swimming stage which penetrates the definitive host). The cercariae start shedding from the snail 4–6 weeks after infection, and back into fresh water (guided by light). In humans, infection occurs by contact with fresh water contaminated by infective cercariae during domestic, occupational or recreative activities (King and Dangerfield-Cha 2008); cercariae are attracted to shadows, warmth and turbulence, e.g. human movement. Upon contact, cercariae penetrate the intact skin, and shed their fork-like tails (the heads are now called schistosomulae). Once inside the host, they migrate via the venous circulation, through the lungs and then the left side of the heart, and into the liver where they mature. Separate sexed adult worms then pair off and migrate via the portal vein system to the mesenteric veins of the intestines (intestinal forms), or the venous plexus of the bladder (*S. haematobium*) where sexual reproduction results in the female laying eggs. The eggs secrete proteolytic enzymes that enable them to migrate to specific sites (the bladder for *S. haematobium* or the intestines for other species) to be passed via the urine or faeces. At this point, schistosome eggs can be detected by direct microscopic examination of the urine or faeces. Pathology from schistosome infection results mostly from host immune reactions to trapped eggs attempting to migrate through to the intestinal or bladder lumen (Colley *et al.,* 2014). Migration of eggs also causes epithelial damage that leads to detectable blood in urine and faeces. Adult schistosomes can live for as long as 30 years

with one schistosome pair producing up to 600 billion schistosome eggs (Basch 1991, Ross *et al.,* 2002, Gryseels *et al.,* 2006).

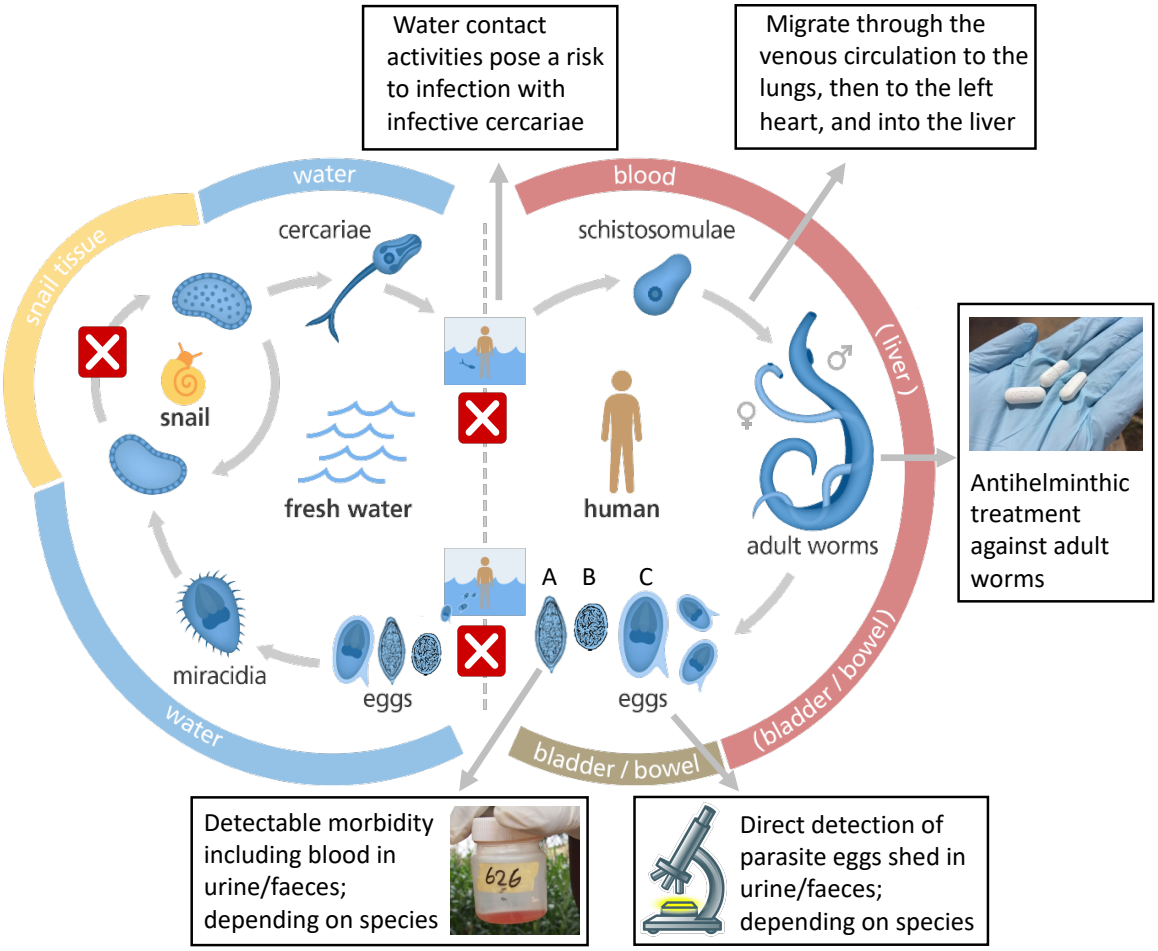

Figure 1.1: Schematic diagram of the schistosome life cycle and its clinical relevance
The figure shows the infective water (specific fresh-water snail intermediate host stage) and the human host stages of the life cycle. The cross signs indicate key points at which the life cycle can be interrupted to break the life cycle and transmission of the parasite. A, eggs of the urinary form *Schistosoma haematobium*; B and C, eggs of intestinal forms *S. japonicum*, and *S. mansoni* respectively. Image adapted from Genome Research Limited.

1.3 Epidemiology

1.3.1 The parasites

As shown in **Figure 1.2a**, the different species of schistosomes that can infect humans have varying geographical distributions. Sub-Saharan Africa alone accounts for more than 90% of global schistosomiasis cases (Colley *et al.,* 2014, World Health Organization 2020b). *Schistosoma haematobium* alone (which this thesis focuses on) accounts for about two-thirds of cases and has been reported in 54 countries. It is the most common species occurring in sub-Saharan Africa and the Middle East (McManus *et al.,* 2018, World Health Organization 2020b). A recent outbreak of urogenital schistosomiasis was however reported in Corsica, France (Boissier *et al.,* 2016). *Schistosoma mansoni* is endemic in sub-Saharan Africa, the Middle East, Brazil, the Caribbean islands, Puerto Rico, Suriname and Venezuela (Colley *et al.,* 2014, World Health Organization 2020b). *Schistosoma japonicum* (zoonotic) in the past, was endemic in Japan (Rollinson *et al.,* 2013), but now in China, the Philippines and Indonesia. With a much lower global prevalence, *S. guineensis* and *S. intercalatum* occur in Central and West Africa, and *S. mekongi* (zoonotic) occurs in the southern parts of Cambodia (Mekong river in Lao People's Democratic Republic) (Colley *et al.,* 2014, World Health Organization 2020b). Some countries including Algeria and India have successfully reduced infection to very low levels, but the WHO is yet to verify whether interruption of transmission has been achieved (see **Figure 1.2b**).

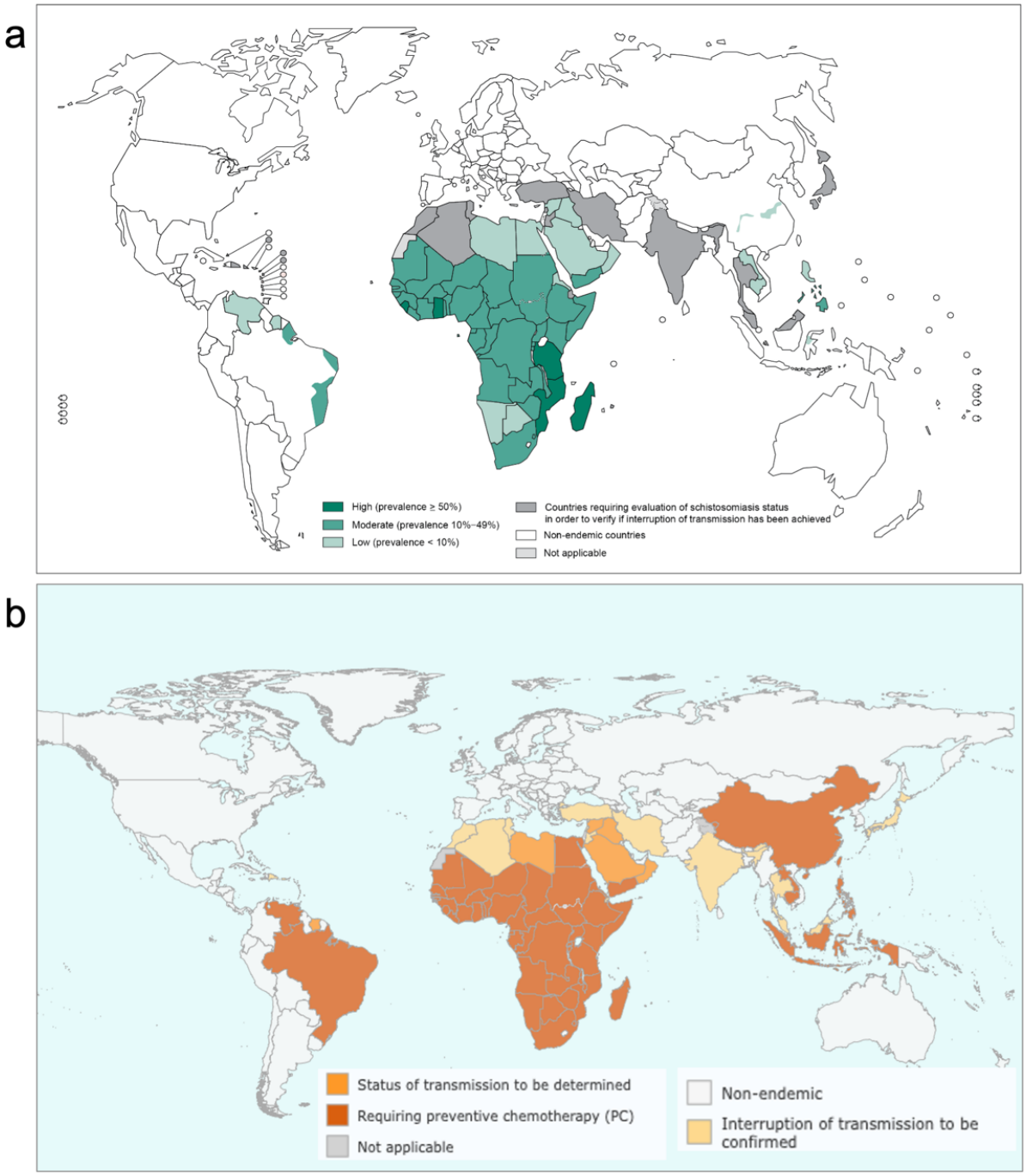

Figure 1.2: Worldwide distribution of schistosomiasis and status of endemic countries
a) Map of the worldwide distribution of schistosomiasis as at 2012. Source from the World Health Organization (WHO) online resource: http://gamapserver.who.int/mapLibrary/[Accessed 12/04/2020]. b) Status of schistosomiasis-endemic countries as at 2018. Source from the WHO online resource https://apps.who.int/neglected_diseases/ntddata/sch/sch.html [Accessed 12/04/2020].

1.3.2 Schistosomiasis in preschool-aged children

In schistosome-endemic areas, a significant amount of the exposure to infection in PSAC is passive, particularly in the youngest children (e.g. use of contaminated water in the home, or children being bathed/sitting in a dish of contaminated fresh water while the guardian conducts domestic chores). Exposure becomes more active as the children grow (e.g. accompanying carers to water sources for domestic chores) (Odogwu *et al.,* 2006, Garba *et al.,* 2010). Therefore, in infants and young children, exposure to infection is closely linked to that of the caregiver. This dissociates as children grow older and become more independent, frequenting contaminated water sources by themselves or with friends and/or older siblings. Although exposure may be dependent on the level of submersion and time spent in infected water, infection is highly likely for PSAC, due in part to lengthened play times (Mduluza and Mutapi 2017). Evidence of exposure in PSAC as observed from field studies by our research group, Parasite Immuno-epidemiology Group is shown in **Figure 1.3**.

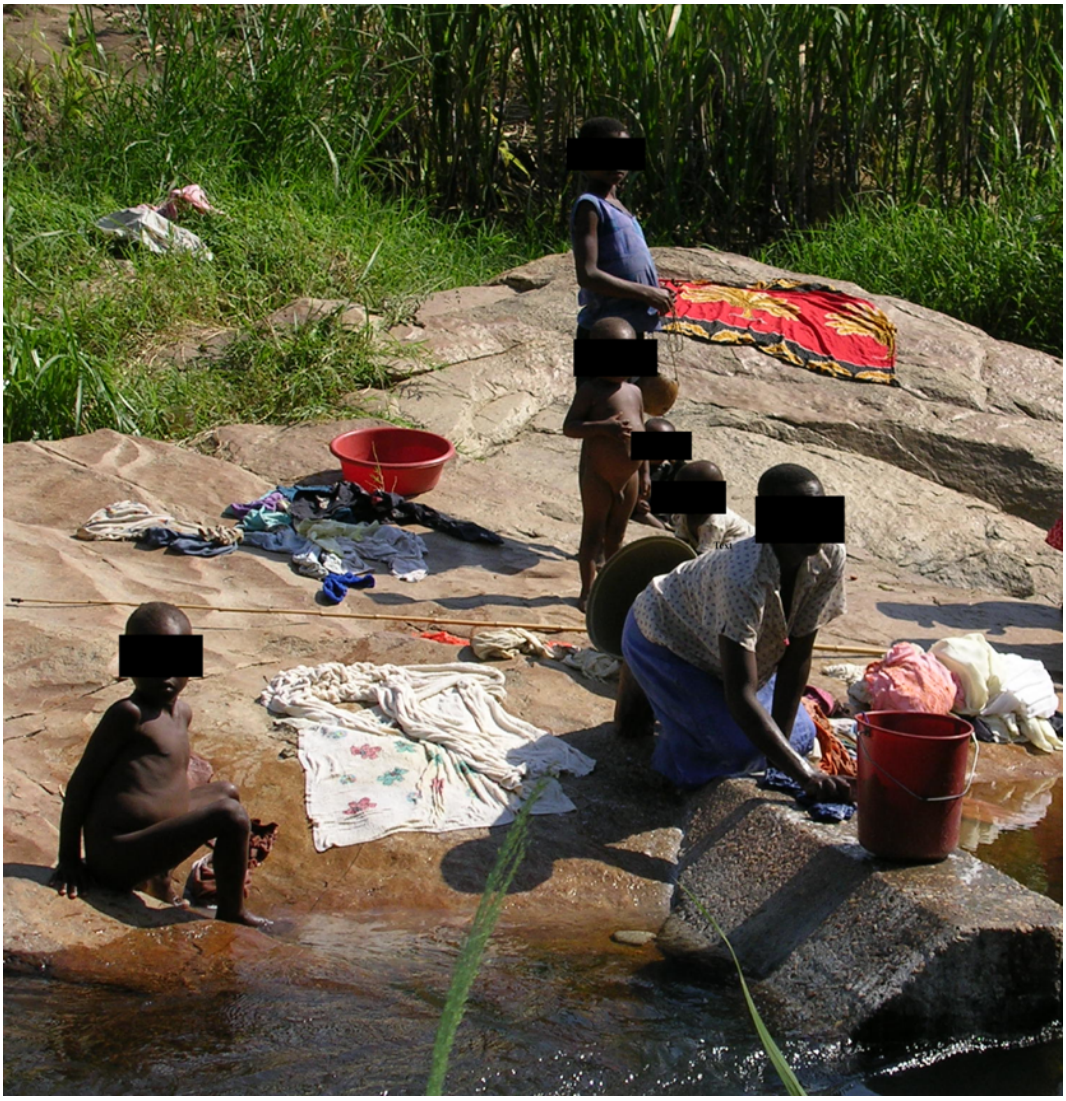

Figure 1.3: Evidence of exposure to schistosome infection in preschool-aged children (PSAC) Children accompany the caregiver to a water contact site, playing while she performs her daily chores. Source: Observations from field work conducted by our research group, Parasite Immuno-epidemiology Group, in schistosome-endemic communities in Zimbabwe. Credit: Prof. Mark Woolhouse.

Exposure to infection is cumulative, and almost all children in high transmission areas will have been exposed to schistosome cercariae by age one, with infection prevalence and intensity increasing with age (Woolhouse *et al.,* 2000, Wami *et al.,* 2014). Thus, there is a need for inclusion of PSAC in large-scale projects mapping the distribution of schistosomiasis. This will inform planning for drug procurement and operational strategies for including these children in national control programmes. In addition to the lack of global estimates of

schistosome infection and disease burden in PSAC, there is a paucity of incidence data in this age group. The work in **Chapter 3** of this thesis contributes to addressing these gaps, using a longitudinal study tracking the incidence of the first schistosome infection (*S. haematobium*) and associated health impacts at an early age.

1.4 Risk factors for schistosome infection

One outstanding feature of schistosomiasis is the focal distribution of infection prevalence and intensity (Gryseels *et al.,* 2006). Several factors influence the risk for schistosome infection in PSAC, including those already identified for other age groups (see **Figure 1.4**). Environmental factors including higher temperature, seasonal rainfall patterns, and high altitude favour parasite development in the intermediate host snail as well as survival of the snail host itself, affecting the force of infection/transmission (Yang *et al.,* 2009, Koukounari *et al.,* 2011, McCreesh *et al.,* 2015). Exposure patterns of the human host are also affected by climatic changes, e.g. hotter seasons encourage increased recreational use of infected water sources; this varies in both the surface area exposed as well as the duration and frequency of exposure (Rudge *et al.,* 2008).

Susceptibility/resistance to (re)infection is affected by the development of acquired immunity, which in turn is a function of the history of infection (Woolhouse *et al.,* 1991). Epidemiological studies of both *S. haematobium* and *S. mansoni* infection, show that the history of antihelminthic treatment also affects the rate of development of acquired protective immunity (Mutapi *et al.,* 1998, Mutapi 2001, Black *et al.,* 2010a, Mitchell *et al.,* 2012, Rujeni *et al.,* 2013). Various social factors have an indirect impact on infection transmission, through activities that contaminate water sources and favour infection transmission (Yang *et al.,* 2009, Ugbomoiko *et al.,* 2010, Ayalew *et al.,* 2011). The relative contribution of these factors in PSAC however remains to be determined. A schematic diagram of various risk factors that influence schistosome infection in PSAC is shown in **Figure 1.4**.

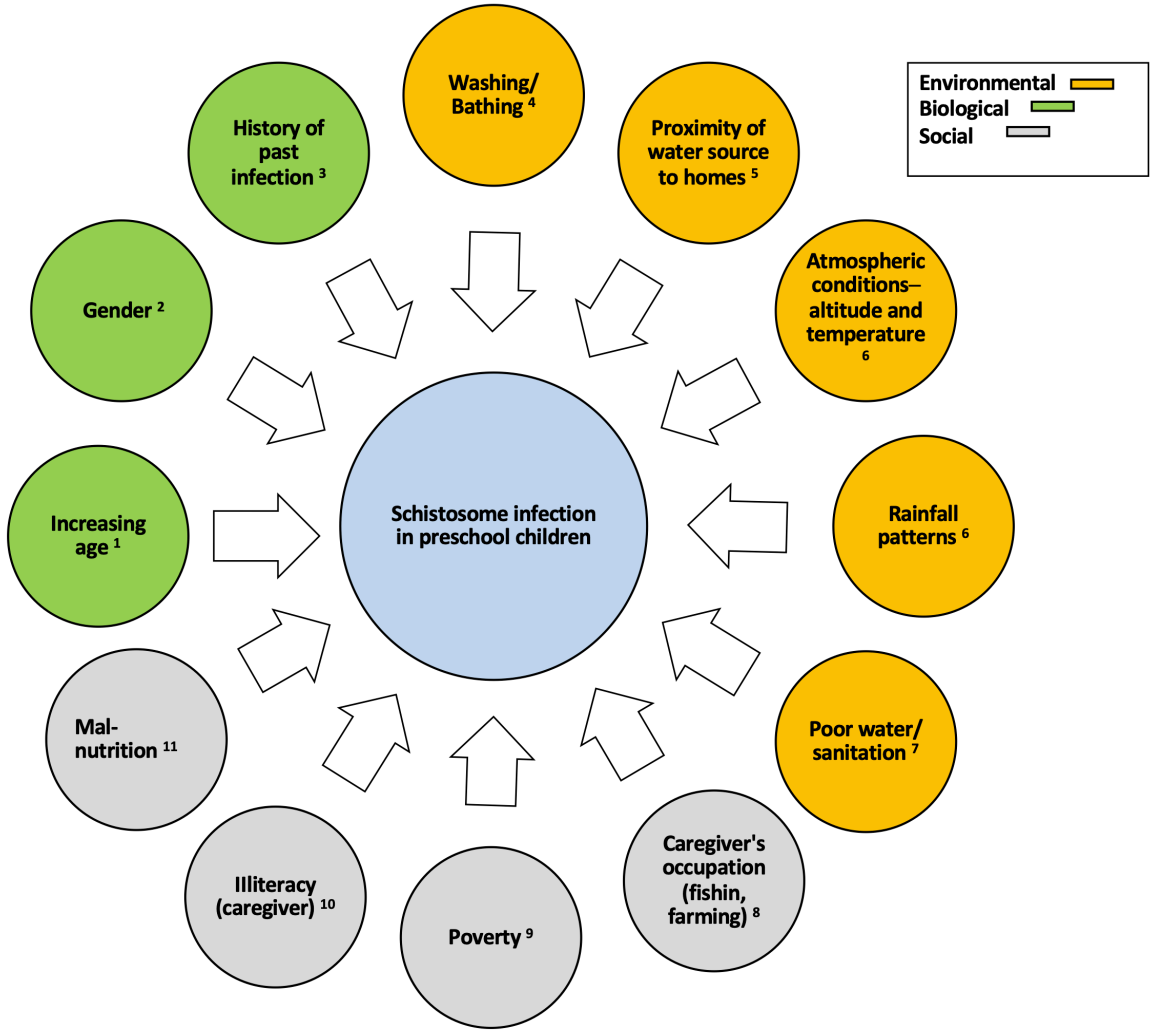

Figure 1.4: Risk factors for schistosome infection in preschool-aged children (PSAC)
Sources from: 1 (Yang *et al.,* 2009); 2 (Rudge *et al.,* 2008, Yang *et al.,* 2009); 3 (Kapito-Tembo *et al.,* 2009); 4 (Rudge *et al.,* 2008), 5 (Rudge *et al.,* 2008, Ugbomoiko *et al.,* 2010); 6 (Yang *et al.,* 2009, Koukounari *et al.,* 2011); 7 (Pruss *et al.,* 2002); 8 (Yang *et al.,* 2009); 9 (Ugbomoiko *et al.,* 2010); 10 (Ayalew *et al.,* 2011); 11 (Hall *et al.,* 2012a).

In addition to these well-studied risk factors, the scientific community is beginning to appreciate the role of the human gut microbiome on schistosome infection and disease (Saad *et al.,* 2012, Dubourg *et al.,* 2013, Glendinning *et al.,* 2014). The gut microbiome plays a vital role in maintaining barrier integrity, and subsequently, impacts the host immune system (Klose and Artis 2016) and explains some of the observed differences in infection patterns across populations (Zupancic *et al.,* 2012). The gut microbiome is believed to be particularly variable

in the early years of life (Kay *et al.,* 2015, Rodriguez *et al.,* 2015), and infections that PSAC are exposed to may influence the structure (abundance and diversity) and function of the microbiome. Recently, a significant difference in the microbiome structure between *S. haematobium*-infected and uninfected Zimbabwean children aged 1 to 10 years was reported (Kay *et al.,* 2015). The direction of this schistosome–microbiome relationship, influence by other factors, and the mechanistic pathways of this effect are yet to be elucidated. Of importance in this age group is the impact of gut microbiome dysbiosis on nutritional status.

Emerging ideas from initiatives such as the Human Microbiome Consortium suggest that, our focus on PSAC should be to understand how the microbiome functions and influences innate susceptibility to schistosome infection and disease progression (Arumugam *et al.,* 2011). Helminths modulate host immune responses for their survival and this has a negative impact on the host microbiome structure and nutrition (Glendinning *et al.,* 2014), and in turn, children's risk of schistosome infection (Hall *et al.,* 2012b). This provides an avenue for non-invasive manipulation of the host microbiome through diet and supplementation. Further evidence on the systemic impacts of schistosome infection, including host–parasite interactions on the microbiome, metabolic and immune systems are discussed in detail in **Section 1.6**.

At present, schistosomiasis interventions targeting the microbiome remain theoretical, as mechanistic studies to establish causal relationships in PSAC are lacking. In schistosome-endemic areas, malnutrition and undernourishment are significant childhood problems, and studies quantifying the relationship between nutrition and the gut microbiome are needed. In addition, there are no studies detailing the attributable fraction of malnutrition due to schistosomiasis or importantly, the impact of schistosomiasis treatment on these factors. Findings in **Chapter 3** of this thesis establish the role or relevance of anthropometric measures as a tool for identifying *S. haematobium*-related nutrition and growth disorders, and identifies the independent risk to nutritional disorders from *S. haematobium* infection in PSAC. The

work in **Chapter 6** also characterises the gut microbiome in PSAC and relates it to *S. haematobium* infection and anthropometric measures of malnutrition and growth.

1.5 Schistosome-specific immune responses and clinical relevance

The interest in immunity in schistosome infections is two-fold. First, hosts in endemic areas develop protective acquired immunity against reinfection, and second, the severe and chronic clinical manifestations of schistosomiasis are immune-mediated. Our understanding of the immune responses to human schistosome infection has been largely informed by experimental models (Colley and Secor 2014). These have given insights into the development of schistosome-specific immune responses mediating resistance to reinfection and pathology, as well as the aetiology and clinical manifestations of morbidity and immunopathology (Colley and Secor 2014). However, the paradigms presented by these studies are not easy to extrapolate to PSAC, due to differences in the natural history of infection. For instance, while a single exposure to cercariae can result in infection in mouse models, human infection in endemic areas is more likely due to repeated exposure (Cheever *et al.,* 2002). Immunology studies in animal models are also limited in their inability to recapitulate morbidity and pathology occurring in the natural human host. For instance, eggs implanted in mice were recently used to develop an experimental model for urogenital schistosomiasis pathology (Fu *et al.,* 2012), but the relevance of this to the human disease forms in young children is still unclear.

Human schistosome immunology is complex and is compounded by numerous heterogeneities across individuals. Nonetheless, some strong patterns have emerged across populations. In brief, schistosome-specific immune responses are mediated by humoral and cellular responses initiated after antigen interaction with antigen-presenting cells (APCs), e.g. dendritic cells, which direct T-cells towards T-helper type 1 (Th1), T-helper type 2 (Th2), or regulatory T-cell (Treg) phenotypes, influencing disease sequelae (Colley *et al.,* 2014, Babu and Nutman

2019). Th1 immune responses are pro-inflammatory and are responsible for killing intracellular parasites and autoimmune responses. Th2 responses on the other hand are more anti-inflammatory and are capable of counteracting the pro-inflammatory effects of Th1 responses (Berger 2000, Spellberg and Edwards 2001). Immune responses to schistosome infection is thus dependent on a complex balance between Th1 and Th2 responses, as well antibody-mediated immunity (Babu and Nutman 2019).

1.5.1 Acute stage

In individuals exposed to schistosomes for the first time, innate immune responses to dying or dead larvae after cercarial penetration of the skin, leads to hypersensitivity (commonly Arthus-type and delayed hypersensitivity) reactions. This can cause a maculopapular pruritic reaction called cercarial dermatitis, at the site of penetration (McManus *et al.,* 2018). In endemic areas however, the acute stage of schistosomiasis, including the symptomatic stage, Katayama fever (the systemic hypersensitivity response to migrating schistosomula or the start of egg deposition) (Ross *et al.,* 2007), receive very little research interest or clinical intervention, compared to non-endemic areas (BMJ Best Practice 2016). It has been suggested that the acute stage of schistosomiasis is rarely observed in endemic areas due to in-utero de-sensitisation to schistosome antigens in infants born to infected mothers, and from repeated exposure from living in such areas (Ross *et al.,* 2007, Sanin *et al.,* 2015, McManus *et al.,* 2018). However, Katayama fever is not only restricted to primary infections (Ross *et al.,* 2007), and early exposure and infection at the preschool age warrant elucidating acute schistosomiasis in PSAC. The immune responses occurring in the early stages of infection are poorly documented in humans, and elucidating these events in PSAC would provide useful information on the nature and development of both pathological and parasite-specific protective immunity.

During the early stages of infection, Th1 cells (CD4+ T-lymphocytes) produce tumour necrosis factor (TNF), interferon gamma, interleukin (IL)-1, IL-2, and IL-6. These are linked with a Th1 response that is crucial for a pro-inflammatory response in the acute stage (Olveda

et al., 2014). Immunological studies of peripheral blood mononuclear cell supernatants from acute schistosomiasis patients have demonstrated the production of these cytokines (de Jesus *et al.,* 2002). The Th1 response is important for protection against infection, and evidence suggests that this response is dampened in chronic long-standing infections and reinfections (Babu and Nutman 2019).

At about 6 weeks post-infection when schistosome worms mature and start producing eggs, Th1 responses begin to decrease, marked by the emergence of a Th2 response (Pearce and MacDonald 2002). One of the mechanisms includes the role of innate immune system components such as epithelial cells, dendritic cells, and macrophages in inducing Th2 cytokines in helminth infection (Babu and Nutman 2019). For example, tissue destruction as a result of schistosome infection leads to the secretion of cytokines and alarmins from epithelial cells, including IL-1, IL-25, and IL-33. These act on natural helper cells (a new population of innate lymphocytes; see Moro *et al.,* (2010)) to induce Th2 cytokines including IL-5 for eosinophilia and IL-13 for goblet cell hyperplasia, both of which are important in the early phase of the anti-helminth response. Cytokines IL-25 and IL-33 also induce the release of IL-4 from basophils and mast cells (Hammad and Lambrecht 2015, Babu and Nutman 2019). In addition, egg deposition can also prime the shift in immune responses from Th1 to Th2. For example, schistosome egg antigens interact with dendritic cells, partially through the action of omega-1 carbohydrate epitopes, for Th2 priming (Everts *et al.,* 2012). Overall, the Th2 polarisation downregulates effector functions of pro-inflammatory mediators, suppresses Th1 promoting cytokines, and primes the body for an established Th2 response in the chronic stage (Babu and Nutman 2019).

1.5.2 Chronic stage

When infection is left untreated, egg antigens stimulate a greater Th2 response, mediated by IL-10. This contributes to granulomas and fibrosis (Cheever *et al.,* 2000), also mediated by IL-13 (Lee *et al.,* 2001). Th2 cells secrete the cytokines IL-4, IL-5, IL-10 and IL-13,

accompanied by high production of eosinophils (stimulated by IL-5), all of which contribute to granuloma formation (Olveda *et al.,* 2014). The Th2 response performs a dual function by promoting host survival, as well as allowing chronic infections to become established and persist to promote disease transmission (Brunet *et al.,* 1997). A review of the nature of immune responses from experimental studies, as relates to human schistosomiasis, suggests that these processes occur after about 12 weeks post-infection (Pearce and MacDonald 2002, Colley and Secor 2014).

Schistosomes have mechanisms for evading and modulating the immune system to promote their long-term survival. *In vitro* studies show that one main mechanism is the ability for schistosome worms to use their somatic stem cells to regenerate their outer tegument, and to incorporate host proteins into their outer surface to reduce their antigenicity and escape immune recognition (Collins *et al.,* 2013). Schistosomes weaken immune attacks by compromising complement function, degrading immunoglobulins, or mimicking and neutralising host immune molecules (Colley and Secor 2014). The worms downregulate immune response by inducing Tregs or anti-inflammatory cytokines (IL-10 and transforming growth factor beta (TGF-β)). The parasites can also utilise host-released cytokines such as IL-5, IL-6 and IL-7 for their growth, development and migration (Maizels and Yazdanbakhsh 2003).

When infection remains untreated, the late chronic stages of infection, especially in the teenage years, is characterised by a gradual decline in the worm burden, as partial immunity begins to develop and worms from early infections begin to die naturally (McManus *et al.,* 2018). The egg burden and associated granulomas begin to reduce, and granulomas are then replaced by fibrous tissue and symptoms become less severe.

1.5.3 Natural and drug-induced protective immunity

Protective immune responses to schistosomes develop slowly, over about 10–15 years (Woolhouse *et al.,* 1991), contributing to the distinctive age-related patterns in infection and immunity, where adolescents and adults are more resistant to infection, compared to PSAC (Mutapi *et al.,* 1997, Woolhouse *et al.,* 2000, Mitchell *et al.,* 2011, Appleby *et al.,* 2012). This is supported by findings from a sero-epidemiological study, which showed that the diversity and intensity of parasite-specific antibodies in serum increased with age and duration of exposure, peaking in the oldest individuals in the study who were 18 years old (Mutapi *et al.,* 2008). Explanations for this include the fact that the immunomodulatory mechanisms induced during schistosome infection, contribute to a delay in the development of protective immunity against schistosomes (McManus *et al.,* 2018). In addition, the hypothesis that partial immunity develops in the host after worms begin to die naturally after about 3–10 years, releasing antigens that can cross-react with antigens of schistosome larvae and stimulate protective immunoglobulin (Ig)E responses, also partly explains this delay (Fitzsimmons *et al.,* 2012). Modelling studies however suggest that the gradual accumulated exposure to antigens from other life-cycle stages over several years also contributes to this observation, without the role of dying worms (Mitchell *et al.,* 2011). These explanations would imply that PSAC could not mount a protective immune response to schistosomiasis, but this has been shown to not be the case if children are treated for schistosome infection (as described below).

One of the consequences of the previous schistosome treatment guidelines was that studies on the effects of PZQ were focused on those targeted by treatment, i.e. SAC and adults. Thus, the immunological consequences of PZQ treatment in PSAC remain poorly characterised. Studies in older children (7 years and above) and adults have shown that the death of adult schistosome worms by treatment with PZQ, enhances schistosome-specific immune responses (Mutapi *et al.,* 2005, Bourke *et al.,* 2013, Nausch *et al.,* 2015, Schmiedel *et al.,* 2015). This leads to (i) removal of the immuno-suppressive effects caused by the adult worms (Mitchell *et al.,*

2012, Nausch *et al.,* 2015, Schmiedel *et al.,* 2015), and (ii) introducing sufficient amounts of parasite-specific antigens to the immune system to reach the threshold required to induce an immune response (Mutapi *et al.,* 2005, Mutapi *et al.,* 2008, Bourke *et al.,* 2013). Modelling studies consistent with the field patterns of age-related immunity demonstrate that, protective immunity to schistosome infection is stimulated by the death of adult worms and reduced fecundity (Mitchell *et al.,* 2012). Previous work conducted by our research group has demonstrated the effect of PZQ on enhancing schistosome-specific immune responses in PSAC. The results showed an increase in anti-parasite IgE titres, 6 weeks post-treatment with PZQ, and this was associated with resistance to reinfection (Rujeni *et al.,* 2013). Thus, the immunological effects of PZQ treatment mimics and accelerates the development of naturally-acquired immunity (Mutapi *et al.,* 2005, Watanabe *et al.,* 2007, Nausch *et al.,* 2011). These changes are associated with reduced reinfection rates, and suggest that pre-treatment infection levels may affect the level of protection against reinfection post-treatment (Black *et al.,* 2010b, Bourke *et al.,* 2013).

Evidence from experimental studies suggest that the early timing of antihelminthic treatment may have additional benefits beyond the transient removal of infection, and may also speed up the development of protective immunity more effectively than treating longer-lasting chronic infections (Behnke and Robinson 1985). In addition, proof-of-principle human clinical studies of malaria infection have already demonstrated that the approach of early infection treatment is successful at inducing long-lasting anti-malaria protective immunity (Roestenberg *et al.,* 2009, Roestenberg *et al.,* 2011, Bhardwaj *et al.,* 2016). Therefore, early treatment of the first helminth infection may induce earlier and greater levels of protection against reinfection, by preferentially inducing the effector rather than regulatory responses (van Riet *et al.,* 2007, de Oliveira Fraga *et al.,* 2010a, de Oliveira Fraga *et al.,* 2010b). The rationale behind treating first infections is that chronic infection induces a constant state of anti-parasite Th2 responses, reducing the overall effect of Th1 effector responses required to fight new infections (Malhotra

et al., 2015). This suggests that treatment of the first schistosome infection could have a longer lasting impact on reducing susceptibility to reinfection, and presents the possibility of targeting treatment for maximum benefit in terms of the future health of the child. A recent study showed that a single PZQ treatment can induce resistance to reinfection in PSAC (Wami *et al.,* 2016), suggesting that if treatment of PSAC is optimally timed, then repetitive treatments may be reduced (de Oliveira Fraga *et al.,* 2010a, de Oliveira Fraga *et al.,* 2010b).

In **Chapter 4** of this thesis, evidence on the health benefits associated with treatment of both acute and chronic *S. haematobium* infections in PSAC, in terms of reinfection rates and subsequent new infections is discussed.

1.6 Systemic impacts of schistosome infection and clinical relevance

1.6.1 The gut microbiota in early life: diversity and disease risks

The human gut comprises a diverse ecosystem of microbes, predominantly bacteria, in addition to viruses, fungi and other eukaryotes, collectively known as the microbiota. The assembly of genomes of the gut microbiota, their products and that of their surrounding intestinal environment make up the microbiome (Lukes *et al.,* 2015). Humans rely on the microbiota for essential functions including extracting essential nutrients from food, as a first line of protection from enteric pathogens, and as a mechanism for shaping the immune system (Human Microbiome Project Consortium 2012). However, these core functions are not fulfilled by the same microbiota in all people, with some varying even within the same individual over the course of life (Shafquat *et al.,* 2014). In fact, microbial colonisation of the gut has been hypothesised to begin *in utero* (Collado *et al.,* 2016).

In very young children, the gut microbial population continues to evolve until about age 3–5 years, and with time, the microbiota diversity within an individual increases while that between individuals within the same communities decrease (Yatsunenko *et al.,* 2012). As reviewed by Glendinning and colleagues, several factors influence the structure (abundance

and diversity) of the microbiome from birth (Glendinning *et al.,* 2014) (see **Figure 1.5**). Given the vital role of the human gut microbiota, a window of opportunity exists, during which any alterations in the establishment of the microbiota in early life, either directly or through systemic routes, has implications on long-term health and disease (Laforest-Lapointe and Arrieta 2017). Evidence of the factors influencing early life microbiota and the role of microbiota alterations in disease risks are summarised in **Figure 1.5** below.

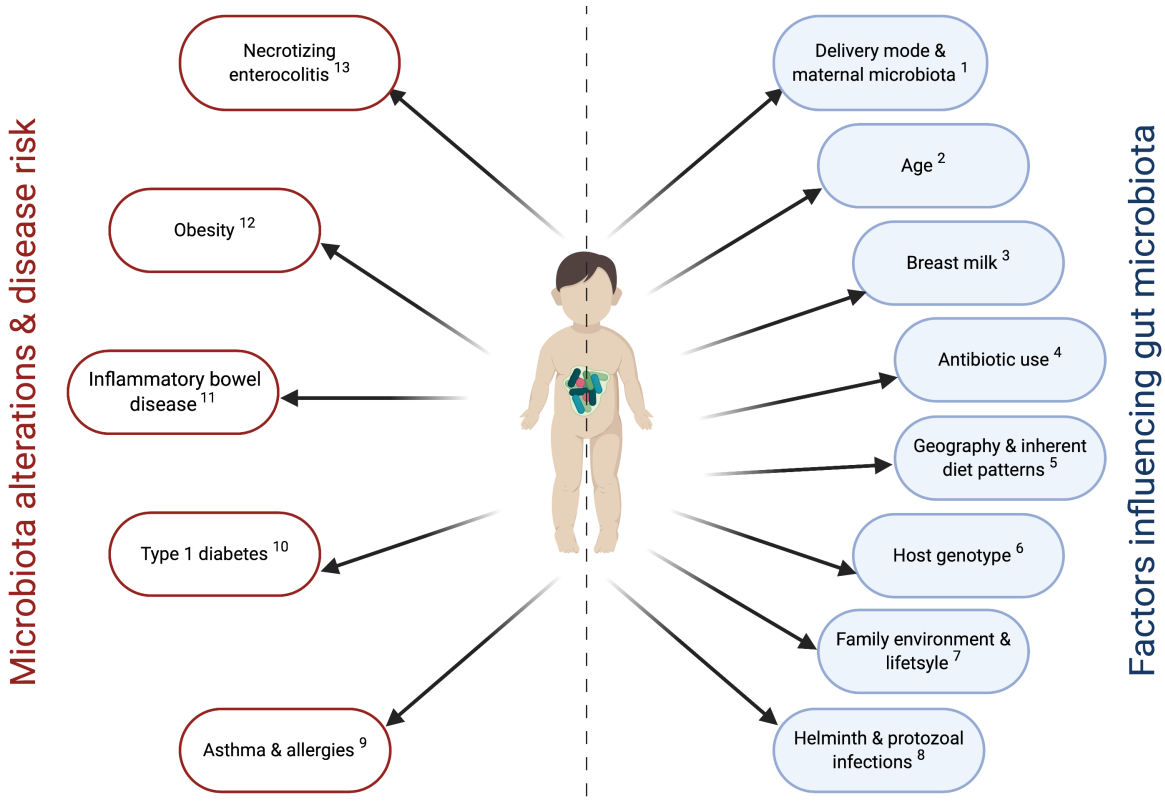

Figure 1.5: Factors influencing the child microbiota diversity, and risk of disease from microbiota alterations
Sources from 1 (Dominguez-Bello *et al.,* 2010), 2 (Yatsunenko *et al.,* 2012, Rodriguez *et al.,* 2015), 3 (Fallani *et al.,* 2010), 4 (Bartosch *et al.,* 2004, Dethlefsen *et al.,* 2008), 5 (De Filippo *et al.,* 2010, Fan *et al.,* 2014, Senghor *et al.,* 2018), 6 (Goodrich *et al.,* 2014), 7 (Martinez *et al.,* 2015) 8 (Mishra *et al.,* 2014), 9 (Bisgaard *et al.,* 2011, Abrahamsson *et al.,* 2014), 10 (Kostic *et al.,* 2015) 11 (Schwiertz *et al.,* 2010, Gevers *et al.,* 2014), 12 (Bervoets *et al.,* 2013, Riva *et al.,* 2017) 13 (Hosny *et al.,* 2017).

1.6.2 Helminth–microbiota interactions

Helminths occupy different host niches for all or part of their life cycle, and play important roles in human health (Glendinning *et al.,* 2014). Nematodes such as the soil-transmitted helminths (STH) *Trichuris trichiura*, (whipworm), *Ascaris lumbricoides* (round worm), *Necator americanus* (hookworm), and *Ancylostoma duodenale* (hookworm) occupy the human intestinal tract (Hotez *et al.,* 2008). Schistosomes however occupy the mesenteric veins of the intestines (*S. mansoni*) and the venous plexus of the bladder (*S. haematobium*) (Gryseels *et al.,* 2006). Enteric helminths occupying such different niches may affect the structure of intestinal microbial communities through multiple parallel routes, either through direct or systemic interactions (Brosschot and Reynolds 2018). For instance, *Lactobacilli* species capable of inducing host regulatory responses (Wells 2011), have been shown to increase during helminth infection in animal models (Plieskatt *et al.,* 2013, Reynolds *et al.,* 2014, Kreisinger *et al.,* 2015). The evidence of such mutually beneficial relationships and the characteristic regulatory response associated with helminth infections, is demonstrated by the fact that *Lactobacilli* species can promote the persistence of helminth infection (Walk *et al.,* 2010, Reynolds *et al.,* 2014). The magnitude, direction, and taxonomic specificity of such helminth-elicited changes in the host can vary due to factors including the helminth species and size, niche specificity, infection intensity, as well as baseline socio-demographic and physiological differences between host individuals (Brosschot and Reynolds 2018). Much of our understanding of the helminth interactions with the gut microbiota in humans has been facilitated by experimental studies, and studies from natural human infections are limited.

Direct interactions that result in helminths modifying the gut microbiota occupying the same niche as they are have been shown. The excretory-secretory products of helminths have been demonstrated to have antimicrobial properties that can modify microbiota composition, as revealed by animal models of *Heligmosomoides polygyrus* (Hewitson *et al.,* 2011) and *Trichuris suis* (Abner *et al.,* 2001). In addition, changes in intestinal mucus dynamics during

helminth infection could favour the growth or otherwise of select microbiota species, as reviewed by Hooper *et al.,* (2012).

However, the influence of helminths on the microbiota does not necessarily occur only when helminths occupy the same niche as the microbiota, and indirect systemic interactions may occur. For example, although primarily occupies the bile ducts, the helminth *Opisthorchis viverrini* induces alterations in the gut microbiome of hamsters (Plieskatt *et al.,* 2013).

1.6.2.1 Schistosome–microbiota interactions

For schistosome infections where adult worms primarily occupy blood vessels, a parallel of the indirect systemic interaction with the gut microbiota is likely to occur. Both experimental and human studies measuring host metabolites associated with the gut microbiota have indicated significant changes in the gut microbiota during schistosome infection (Wang *et al.,* 2004, Balog *et al.,* 2011). Recently, fluctuations in the composition of the gut microbiota of mice infected with *S. mansoni*, before and after intestinal damage from egg transmission was shown (Jenkins *et al.,* 2018). Furthermore, the absence of microbiota during schistosome infection in mice results in diminished faecal egg excretion (Holzscheiter *et al.,* 2014).

The factors underlying the indirect effects of schistosome infections on the microbiota are dependent on the Th2 immune responses elicited during helminth infections. A case of microbiota-dependent immune modulation includes the upregulation of the host antimicrobial peptide, angiogenin-4, during murine *T. muris* (D'Elia *et al.,* 2009, Forman *et al.,* 2012) and *Nippostrongylus brasiliensis* (Datta *et al.,* 2005, Fricke *et al.,* 2015) infection, which is associated with Th2 immune responses and shifts in microbiota composition. This is consistent with the observation that exogenous administration of IL-25, a factor for initiating Th2 immune responses in the absence of live helminth infections (Saenz *et al.,* 2008), replicates the host antimicrobial peptide expression and shifts in microbiota composition phenotype observed in mice infected with *N. brasiliensis* (Fricke *et al.,* 2015). The increased production

of IL-22 during human helminth infections (Broadhurst *et al.,* 2010) also functions as a cytokine mediator between the gut microbiota, the intestinal mucosa, and host immunity (Leung and Loke 2013). Mouse and *in vitro* studies have identified that this occurs by IL-22 stimulating the production of antimicrobial peptides such as the β-defensins (Liang *et al.,* 2006, Wolk *et al.,* 2006), as well as maintaining mucosal barrier homeostasis by promoting inflammation, tissue repair, and reduced dissemination of intestinal commensal bacteria to the spleen and liver (Sonnenberg *et al.,* 2011). The overall effects of Th2 immune responses on increased antimicrobial peptides, IL-22, and mucus production, is in line with the need for host tissue repair post-infection (Sonnenberg *et al.,* 2011, Turner *et al.,* 2013, Peterson and Artis 2014); IL-22 during schistosome infection has been shown to protect the host against egg-induced fibrosis and cirrhosis in humans (Sertorio *et al.,* 2015), and from granulomas *in vitro* (Nady *et al.,* 2017). Furthermore, schistosome worms have direct access to host blood flow and nutrients, and hence utilise large amounts of host glucose (Bueding 1950). Utilizing large amounts of the host's glucose compromises epithelial glucose absorption, as revealed by *H. polygyrus* murine models (Shea-Donohue *et al.,* 2001). Thus, the reduced availability of intestinal nutrients to resident microbiota may be another plausible explanation for the significant impacts of schistosome infection on the host microbiota.

1.6.2.2 Helminth–microbiota interactions and immune homeostasis

Although schistosomes can directly evade and modulate the immune system (as described earlier in **Section 1.5.2**), the effects of schistosome infection on the gut microbiome can indirectly influence immune responses, as reviewed for helminth infections (Leung and Loke 2013). Differences in gut microbiota composition have been associated with the differentiation of Tregs (Gaboriau-Routhiau *et al.,* 2009, Atarashi *et al.,* 2011, Atarashi *et al.,* 2013) and mucosal Th17 cells (Gaboriau-Routhiau *et al.,* 2009, Ivanov *et al.,* 2009), as well as susceptibility to allergies (Reynolds and Finlay 2013) and inflammatory bowel disease (Nell *et al.,* 2010). The role of increased Treg populations from gut microbiota differences, in the

production of helminth-associated regulatory cytokines such as IL-10, independent of Th2 immune responses has been shown in mice (Anderson *et al.,* 2007, Rubtsov *et al.,* 2008); its role in the production of other regulatory cytokines like TGF-β has also been reviewed (Reynolds *et al.,* 2012, Reynolds and Maizels 2012). These suggest that the helminth interactions that result in microbiota alterations can influence host immune homeostasis, and thus differences in infection and disease patterns.

Furthermore, the microbial population in the intestinal tract provides colonisation resistance against concurrent infections, and the relationship between the microbiota and susceptibility or resistance to infection has been extensively reviewed (Kamada *et al.,* 2013, Vogt *et al.,* 2015). A loss or increase in abundance of specific microbial taxa can contribute to susceptibility to infectious diseases (Kamada *et al.,* 2013), growth and/or virulence of enteric pathogenic bacteria (Vogt *et al.,* 2015), and to the observed differences in infection and disease patterns across human populations (Zupancic *et al.,* 2012).

In the case of schistosomiasis, there is an age-dependent infection pattern, with the gradual development of protective immune responses with age (Woolhouse 1998). Given the helminth-associated shifts in microbiota and immune responses described above, the time of helminth infection relative to the establishment of the gut microbiota in early life may be an important determinant of overall health outcome, especially in PSAC whose microbiota are still evolving.

1.6.3 Schistosome–metabolism interactions

Historically, schistosome worms have been thought to adapt to the definitive host's environment. In addition to the ability to evade attack from the host's immune system, schistosome worms can exploit host endocrine, metabolite and immune factors for survival (Halton 1997). Like for schistosome–microbiota interactions, much of the knowledge on the effects of schistosome infection on host metabolism is from experimental models, and human

studies showing such interactions are limited. Experimental studies have shown that schistosome worms require host-derived endocrine steroid and thyroid hormones in order to establish themselves and survive (de Mendonca *et al.,* 2000, Saule *et al.,* 2002, You *et al.,* 2015). Without such host factors available to schistosome worms, there are parasite-specific morphological abnormalities including poor growth and reduced fecundity, as well as attenuated granuloma formation in host liver (Cheng *et al.,* 2008, Lamb *et al.,* 2010, Tang *et al.,* 2013). The findings from the studies described above suggest evidence of the direct host–parasite interactions that lead to schistosome worms manipulating the host metabolism.

Schistosome worms reside directly in blood vessels close to the intestine or urinary bladder, with direct access to the flow of nutrients. Experimental studies demonstrate that schistosome worms import glucose across their body surface and through the gut (Wu *et al.,* 2010b), consuming their dry body weight's worth of glucose every 5–6 hours (Bueding 1950). In addition, the liver injury caused by schistosomes during murine infection (Wu *et al.,* 2010b) is marked by stimulated host glycolysis, manifested by reduced plasma glucose as well as reduced glucose and glycogen stores in the liver (Wang *et al.,* 2004). This is supported by studies in *S. japonicum*-infected mice, indicating the high expression of genes involved in catabolism (glucose uptake, glycolysis and fatty acid oxidation), and the suppression of genes involved in anabolism (glycogen synthesis) in the liver (Xu *et al.,* 2019). Schistosome worms have long been known to also lack the ability to synthesize *de novo* sterols, fatty acids and purines, which they require for growth and development (Levy and Read 1975a). The *in vitro* evidence available suggests that the worms resort to the uptake of host purine (Levy and Read 1975b) and lipid molecules (Meyer *et al.,* 1970).

The Th2 immune responses elicited during schistosome infection can also alter host metabolic function, disease patterns, and overall health (Brestoff and Artis 2015). Studies show that the prolonged production of cytokines IL-10, IL-4, and interferon-gamma are associated with a reduced likelihood of type 1 diabetes in mice (Zaccone *et al.,* 2003). Some of the mechanisms

observed from experimental studies include the ability of helminth-induced adipose tissue eosinophilia to enhance glucose tolerance and alleviate insulin resistance (Wu *et al.,* 2011). Taken together, the evidence from experimental (Zaccone *et al.,* 2003, Stanley *et al.,* 2009, Wu *et al.,* 2010b) and limited human studies (Chen *et al.,* 2013, Wolde *et al.,* 2019) suggest that, chronic helminth infection and its impact on reduced host glucose and lipids may have the potential to prevent or delay the occurrence of the metabolic syndrome and related dysfunctions (van der Zande *et al.,* 2019, Wolde *et al.,* 2019).

There is also current evidence as reviewed by Rowland *et al.,* (2018), that the gut microbiota produce and utilize gut metabolites, and have profound effects on regulating host metabolism. Therefore, the overall parasite-elicited metabolic alterations can be thought of as the combined result of altered host, microbiota, and helminth production of metabolites, as well as altered host utilization, excretion, and absorption of metabolites.

1.6.4 Impact of host–parasite interactions on host nutrition

Given the vital role of the microbiome and metabolism on host nutrition and overall health, the systemic schistosome–microbiota–metabolism interactions may thus play a role in the aetiology of childhood malnutrition (Kane *et al.,* 2015).

The direct alteration of host metabolism, uptake of essential nutrients and metabolites by schistosome worms, and the increased energy demands on the host (as outlined in **Section 1.6.3**), contribute to reduced nutrient availability, and subsequently the malnutrition and growth-related sequelae observed in children with schistosomiasis (Freer *et al.,* 2018). The gut microbiota produce and utilize gut metabolites (Rowland *et al.,* 2018). In addition, host enzymes alone are incapable of breaking down many complex carbohydrates (Flint *et al.,* 2012). Thus, the helminth-induced changes to the microbiota may alter the extent to which the host receives nutrition. The evidence available for this however suggests that these effects may depend on the physiological state of the host and will vary across gut microbiota species. For

instance, experimental studies have shown that infection with *Trichuris* species is associated with reduced carbohydrate metabolism, which coincided with significant declines in abundance of *Ruminoccocus, Prevotella, Roseburia*, and *Parabacteroides*, all of which are carbohydrate-utilizing bacteria (Li *et al.,* 2012, Lee *et al.,* 2014, Ramanan *et al.,* 2016). Microbiome dysbiosis during helminth infection is also accompanied by a decrease in breakdown products of dietary plant-derived carbohydrates and intermediates involved in the synthesis of amino acids, and a reduction in the detection of breakdown products of haem, leading to weight loss in mice (Houlden *et al.,* 2015).

These findings point to the contribution of such helminth-induced mechanisms to the observed malnutrition and weight loss associated with schistosome infection (Stephenson 1993, Stephenson *et al.,* 2000, Bustinduy *et al.,* 2013, Freer *et al.,* 2018). However, a vast majority of the evidence available for host-helminth interactions are from animal studies, while there are limited human studies on the impact of schistosome infection on host microbiome and metabolism. In particular, the events that occur during early life in PSAC, including infections, have implications on health and disease later in life. Better strategies for schistosome control, including vaccines, will rely on a better understanding of how schistosomes utilize essential host nutrients and molecules for their survival. Further research into the systemic impacts of schistosome infections, especially on the human host microbiome and metabolism, should present opportunities to develop simple non-invasive nutritional interventions targeted at populations at risk of infection. For example, if schistosome infection in PSAC is associated with metabolic and microbiota alterations that are associated with malnourishment and altered immunity, then supplementation would be a useful complementary therapeutic tool to accompany standard PZQ treatment, particularly in PSAC. There is already a global vitamin A supplementation programme for PSAC in tropical countries (World Health Organization 1997), and increasing research in nutraceuticals should inform additional child supplementation programmes in affected countries. Work from **Chapters 5 and 6** in this thesis

characterises the association of *S. haematobium* infection with alterations in host metabolism and gut microbiota composition respectively. It further discusses how such changes are related to disease burden, and the schistosome-associated morbidity observed in PSAC.

1.7 Pathology and morbidity

Schistosome pathology and morbidity are still being defined in older children and adults. For example, urinary schistosomiasis was recently renamed urogenital schistosomiasis to reflect the increasing recognition of the genital manifestations of *S. haematobium*-related disease (World Health Organization 2009). Pathology and morbidity remain poorly described and studied in PSAC, compared to SAC (Odogwu *et al.,* 2006). However, studies are beginning to shed light on schistosome morbidity in this age group. For instance, a recent study using ultrasound has contributed to describing schistosome-associated urinary morbidity, including bladder wall thickening and hydronephrosis in PSAC (Barda *et al.,* 2017).

In PSAC in endemic areas, clinical symptoms upon schistosome exposure and infection (e.g. cercarial dermatitis and fever) may go unrecognised (Lambertucci 1993, Bottieau *et al.,* 2006, Gryseels *et al.,* 2006), or be mistaken for other febrile illnesses such as malaria, which present with similar symptoms. In the established and chronic stages of the disease, organ-specific morbidity can develop and this is determined by the type of schistosome species involved (intestinal or urogenital) (King and Dangerfield-Cha 2008). This is mostly caused by host immune reactions to trapped or accumulated parasite eggs in tissues, the development of granuloma and fibrosis, as well as migration of parasite eggs through tissues and into the intestinal or urinary bladder lumen (Pearce and MacDonald 2002, van Riet *et al.,* 2007, McManus *et al.,* 2018). Migrating eggs secrete proteolytic enzymes, which stimulate inflammatory and granulomatous reactions at specific sites (Cheever *et al.,* 2000), and is often accompanied by sloughing, ulceration and bleeding at specific sites. Disease manifestation is however influenced by factors including the nature and magnitude of immune responses (Pearce and MacDonald 2002). Variation in host genetics (Isnard *et al.,* 2011), infection

intensity and egg-patency (Stothard *et al.,* 2011, Colley *et al.,* 2014) and *in utero* sensitization to schistosome antigens (Maizels *et al.,* 2014) give rise to heterogeneities in schistosome morbidity/pathology. Further studies elucidating the relative impact of these factors will be important to inform complementary interventions on reducing schistosome-related morbidity in PSAC. Morbidity associated with schistosomiasis in PSAC is summarised in **Table 1.1**. As this thesis focuses on paediatric schistosomiasis, morbidity described here was limited to this age group. A detailed review of morbidity from schistosomiasis from birth to adulthood can be found in Freer *et al.,* (2018).

Table 1.1: Morbidity associated with schistosomiasis in preschool-aged children (PSAC)

Urinary (*S. haematobium*)	**Intestinal (*S. mansoni, S. japonicum*)**	**Generalized pathology**
Dysuria	Pseudopolyposis	Anaemia
Pollakisuria	Microulcerations	Malnutrition
Proteinuria	Superficial bleeding	Stunting
Ulceration and pseudopolyposis of vesical and ureteral walls	Hepatosplenomegaly	Fatigue
Fibrosis of the bladder and lower ureters	Fibrosis	Poor cognition
Hydroureter and hydronephrosis		
Genital lesions		

Sources: (Cheever *et al.,* 1978, Gryseels and Polderman 1987, Gryseels 1992, Poggensee and Feldmeier 2001, Gryseels *et al.,* 2006, King and Dangerfield-Cha 2008, Colley *et al.,* 2014).

Schistosome infections damage epithelial barriers, and chronic infection results in anaemia, poor nutrition and growth (Bustinduy *et al.,* 2013, Okafor-Elenwo and Elenwo 2014, Guerrant *et al.,* 2016). Thus, even with the low parasite burdens in PSAC, the pro-inflammatory response generated can quickly lead to chronic morbidity (Leenstra *et al.,* 2006). For example, schistosome-associated anaemia and malnutrition in older Kenyan children (5–18 years old) was attributed to pathology beginning at the preschool age (Bustinduy *et al.,* 2013). The nutritional effects of schistosome infection could also be attributed to microbiome dysbiosis and alterations in host metabolism as described in **Section 1.6.4**. Such growth-related morbidities can be accompanied with poor cognition, which is usually seen in the school-going

years (Colley *et al.,* 2014). Thus, the impact of schistosomiasis in PSAC should be an integral part of interventions targeted at improving early child health and development.

It is also possible that schistosome infections have wider impacts on childhood health. In PSAC, chronic prenatal exposure or sensitisation to helminth infection is reported to be associated with reduced efficacy of childhood vaccines, through induction of a persistent Th2 immune response phenotype (Chen *et al.,* 2012, Gebreegziabiher *et al.,* 2014). Chronic exposure is also thought to be associated with environmental enteropathy, which affects the efficacy of vaccines at infancy (Levine 2010). Reduced vaccine efficacy will mean susceptibility to infection and disease from many vaccine-preventable infections. Thus, controlling schistosomiasis in PSAC may have the added health benefit of improving vaccine efficacy, and as such requires appropriate studies to investigate and provide the scientific evidence required to promote policy change.

Poor awareness, recognition, and lack of understanding and quantification of schistosome-associated morbidity had previously made schistosome control in PSAC a lower priority than in older children and adults. Concerted efforts are now beginning to correct this. There is a need for biomarkers of environmental enteropathy in PSAC (Guerrant *et al.,* 2016), mechanistic studies to infer causality, and well-defined predictors of growth and nutrition in PSAC exposed to schistosomiasis. The work presented in **Chapter 3** of this thesis also focuses on the incidence of schistosome-related morbidity from the first schistosome infection, as well as how quickly this presents and can be detected post-infection, using currently available tools.

1.8 Paediatric schistosomiasis in coinfected hosts

Similar to all other age groups in tropical regions, PSAC in schistosome-endemic areas are at risk of coinfections and therefore, comorbidities, some of which may arise later in life. For instance, urogenital lesions from *S. haematobium* infection may remain until puberty (Subramanian *et al.,* 1999), and a compromised urogenital epithelium has been associated with

an increased risk of HIV infection (Kjetland *et al.,* 2005, Wall *et al.,* 2018). Studies in older children (5–18 years) indicate that the presence of multiple parasitic infections in endemic areas lead to significant morbidity, including anaemia and malnutrition, and these are important in the formative years of PSAC (Bustinduy *et al.,* 2013). Specific coinfection studies include malaria–schistosomiasis, where studies in SAC suggest that malaria severity is compounded by schistosome infection, and that malaria treatment is more effective when schistosome infection is treated (Mbah *et al.,* 2014, Getie *et al.,* 2015). However, no detailed studies have been conducted to determine the fraction of malaria deaths in PSAC that are attributable to schistosome coinfection. In another instance, *in vitro* studies have shown that *Salmonella* can evade optimal antibiotic treatment by binding to schistosomes using fimbral proteins (FimH) present on its surface. This can result in significant increases in the *Salmonella* population, and eventually, antimicrobial resistance from repetitive ineffective therapy (Barnhill *et al.,* 2011). Conversely, this interaction can complicate schistosomiasis treatment, as effective therapy could result in the release of *Salmonella*, thereby causing septicaemia (Gendrel *et al.,* 1994). Such findings need validation in PSAC to allow the implementation of appropriate interventions. The significance of such indirect interactions between schistosomes (*S. haematobium*), intestinal bacteria (microbiota) and antimicrobial resistance gene abundance and diversity is also discussed in **Chapter 6** of this thesis.

Considering that schistosome infections induce an immune response, the timing and type of response (i.e. Th1 or Th2) induced may exacerbate or lessen the impact of other infections. For example, chronic sensitisation or exposure to schistosome infection *in utero* (predominantly involving Th2 immune responses) is associated with reduced vaccine efficacy at birth (as this depends on Th1 immune responses), and thus susceptibility to vaccine-preventable infections (Elias *et al.,* 2005, Chen *et al.,* 2012, Gebreegziabiher *et al.,* 2014). *Schistosoma haematobium* infection has been shown to reduce the level of protective IgG responses to malaria vaccine candidates including the merozoite surface protein and

glutamate-rich protein in humans (Courtin *et al.,* 2011). Equally, synergistic responses against schistosome infections are known to cross-protect individuals from infection by other pathogens such as *T. muris* (Pearce and MacDonald 2002), and protect against higher *Plasmodium falciparum* densities (Briand *et al.,* 2005). There is contrasting evidence showing no independent association between schistosome infection or PZQ treatment during pregnancy and vaccine efficacy or immune response to vaccines in infancy (Webb *et al.,* 2011, Malhotra *et al.,* 2015). However, some of the studies on schistosome–vaccine interactions are limited by methodological problems including study design and sample size (Gebreegziabiher *et al.,* 2014), and the use of diagnostic methods such as serology, which can detect past infections that may have no effect on *in utero* exposure (Malhotra *et al.,* 2015). However, such findings are important in the formative years of PSAC. Elucidating the susceptibility or otherwise of PSAC to coinfections in schistosome-endemic areas, and their impact on disease sequelae and relevance to integrated healthcare will be of interest.

1.9 Diagnosis of infection

The current guidelines for the treatment of schistosomiasis in PSAC is that diagnosis is made before treatment, unlike the MDA approach where SAC are treated without diagnosis (World Health Organization 2011, Bustinduy *et al.,* 2016a). This means that in the "diagnose and treat" model where treatment of PSAC will be on a case-by-case basis through regular health services, accurate point-of-care (POC) diagnostics are key to guide the targeted treatment. In addition, diagnosis must be sensitive and specific in relation to prevalence and intensity of infection in PSAC (Inwig *et al.,* 2002, Raslich *et al.,* 2007).

1.9.1 Parasitological techniques

The direct parasitological methods, Kato–Katz (Katz *et al.,* 1972) and urine filtration (Mott *et al.,* 1982) for intestinal and urogenital schistosomiasis respectively, are the conventional diagnostic methods recommended by the WHO (World Health Organization 2020b). These involve the microscopic detection of parasite eggs in stool or urine samples, and are

convenient, specific, rapid, cheap, and suitable for field applications in PSAC (De Vlas *et al.,* 1997, Yu *et al.,* 2007). Details of these methods as used in the diagnosis of schistosome infection in this thesis are described in **Chapter 2**.

Infection intensity increases with age (Woolhouse *et al.,* 2000, Mitchell *et al.,* 2011, Appleby *et al.,* 2012, Wami *et al.,* 2014), hence PSAC usually present with low infection burden, while some may carry only single-sex infections (Stothard *et al.,* 2011, Colley *et al.,* 2014). Parasitological methods therefore have reduced sensitivity in PSAC and lack the ability to detect pre-patent infections (Bergquist *et al.,* 2009, Coulibaly *et al.,* 2013, Wami *et al.,* 2014, Utzinger *et al.,* 2015). Light infections in PSAC may be missed by parasitological techniques, thus underestimating the prevalence of schistosome infection (Le and Hsieh 2017).

Nonetheless, in addition to the already known limitations of low sensitivity of the parasitological methods, these methods have the added operational challenge of obtaining adequate urine and stool samples from PSAC (Stothard 2009). Particularly, egg excretion may show daily, diurnal and within-specimen variation, as well as clumping of eggs in stool, all of which reduce precision and sensitivity (Doehring *et al.,* 1983, Engels *et al.,* 1996, Utzinger *et al.,* 2001). To improve sensitivity, it is recommended to collect replicate samples over consecutive days (Engels *et al.,* 1996), but this can be a challenge in very young children.

1.9.2 Other methods for infection and disease diagnosis

To address the issues of sensitivity, other methods have been developed in the laboratory, and are at different stages of translation into field tools. Immunological tests that employ the detection of antibodies and cytokines, including immunoglobulins (Beck *et al.,* 2008), TNF, interferons, and interleukins (Pearce and MacDonald 2002, Colley and Secor 2014) are more sensitive and can be used in areas with low egg burden (Gonçalves *et al.,* 2006). For instance, immunological detection of schistosome-specific IgM antibodies in sera has been shown to be more sensitive in diagnosing infection in PSAC than parasitological methods (Wami *et al.,*

2014). Issues of cost, cross-reactivity and blood sampling of PSAC however pose challenges (Kanamura *et al.,* 1998, Oliveira *et al.,* 2005). Nonetheless, such tests can be very useful in travellers, and in PSAC and older people with little or no egg excretion (Whitty *et al.,* 2000). It can also aid in understanding evidence of exposure, susceptibility, and immune mechanisms during schistosome infection.

Circulating anodic antigen (CAA) (Feldmeier *et al.,* 1986, Gundersen *et al.,* 1992) and circulating cathodic antigen (CCA) (Coulibaly *et al.,* 2013), are waste products that are released when schistosomes feed on red blood cells (Deelder *et al.,* 1980). The non-invasive detection of these worm antigens in urine as point-of-care (POC)–CCA and CAA tests have also been evaluated in PSAC. They have shown to be a reliable tool in diagnosing *S. mansoni* (Coulibaly *et al.,* 2013, Ochodo *et al.,* 2015), but less so in *S. haematobium* diagnosis (Ashton *et al.,* 2011, Sousa *et al.,* 2019). Models using data in older children have shown that in endemic areas, POC–CCA is a better predictor of infection prevalence than egg-based techniques, especially for post-treatment monitoring (Prada *et al.,* 2018). The enhanced sensitivity of such diagnostics (Kittur *et al.,* 2016) and the logistic advantage of its use on urine instead of more cumbersome samples like stool or blood (Utzinger *et al.,* 2015) will make it a useful tool in PSAC, given that this age group usually presents with low intensity infections (Stothard *et al.,* 2011, Colley *et al.,* 2014). Issues of cost in low-resource settings, as well as limited applicability in low endemicity areas where individuals may have very few worms, would however need to be overcome (Kanamura *et al.,* 2002, McManus *et al.,* 2018). Increasing sample volumes for testing has been suggested as a potential to overcome sensitivity issues, especially with developing accurate POC–CAA for *S. haematobium* diagnosis (Knopp *et al.,* 2015).

The advent of molecular techniques such as polymerase chain reaction (PCR)-based techniques, holds promise of additional diagnostic tools in PSAC (Oliveira *et al.,* 2010, He *et al.,* 2016). Compared to CCA and parasitology, PCR was most effective in detecting low

intensity *S. mansoni* and *S. haematobium* infections (Ibironke *et al.,* 2012, Lodh *et al.,* 2013). The use of cell-free parasite DNA present in urine and saliva (Kato-Hayashi *et al.,* 2013) in PCR techniques can ease sampling difficulties in PSAC and detect early infections (Kato-Hayashi *et al.,* 2010). MicroRNAs have been characterised in animal models of *S. japonicum* (Cheng *et al.,* 2013) and *S. mansoni* (Hoy *et al.,* 2014). Despite conflicting reports on the utility of miR-223 as a biomarker for helminth infections (He *et al.,* 2013, Hoy *et al.,* 2014), microRNAs have great diagnostic potential in PSAC and should be explored further. The drawback of molecular diagnosis is that in the absence of POC versions of these tests, expertise and specialised equipment are required (Pontes *et al.,* 2003), and this poses a challenge in resource-poor settings.

Point-of-care ultrasonography (POCUS) is becoming a popular tool for indirect diagnosis in large-scale trials for schistosomiasis control, to assess organ-specific pathology associated with schistosomiasis (Richter *et al.,* 2000, Akpata *et al.,* 2015). As applied for *S. mekongi* in Cambodia, the effect of schistosomiasis interventions on morbidity can be monitored using this method (Chigusa *et al.,* 2006). Available evidence and experiences of POCUS for detecting schistosome-associated morbidity has been thoroughly reviewed; a simplified WHO protocol to reduce turnaround time and increase its field applicability has been recommended (Bélard *et al.,* 2016).

1.9.3 Operational need for better diagnostics in preschool-aged children

Following current WHO guidelines for PSAC, diagnosis is required before treatment, i.e. a "diagnose and treat" strategy, and not PC (World Health Organization 2011, Bustinduy *et al.,* 2016a). Missing an infection due to insensitive diagnostics will mean missed treatments, and this can have a significant impact on the health of the child, particularly in light of the knowledge that most schistosome infections lead to clinical symptoms and substantial morbidity (Sacko *et al.,* 2011, King 2015). Moreover, untreated PSAC may act as reservoirs

for transmission and infection, consequently hampering elimination efforts. With PSAC presenting with light and/or pre-patent infections, the sensitivity of conventional methods is reduced and infections may be missed (Bergquist *et al.,* 2009, Coulibaly *et al.,* 2013, Wami *et al.,* 2014, Utzinger *et al.,* 2015). Thus, the applicability of current conventional methods as used in SAC and adults in endemic areas may not be the most appropriate in this context. Realistically looking ahead to primary health care for infants and young children in helminth-endemic areas, more useful diagnostics will be POC tests that integrate diagnosis of several conditions/comorbidities, e.g. schistosomiasis, malaria, anaemia.

1.10 Morbidity markers for schistosomiasis

The majority of morbidity biomarkers associated with schistosomiasis are non-specific, relating to physiological, biochemical and immunological changes. As such, their interpretation in the light of coinfections and comorbidities in endemic areas is complex, and calls for better defined schistosome morbidity indicators in PSAC (Webster *et al.,* 2009). Operationally useful morbidity markers should: (i) provide more information than that obtained from parasitological detection of infection (Vennervald *et al.,* 2000), (ii) be measurable in one sample and at any time (Vennervald *et al.,* 2000), (iii) detect early degrees of morbidity (Hatz *et al.,* 1992), and (iv) predict development of severe disease (Vennervald *et al.,* 1998).

The classic urinary markers, haematuria and proteinuria (excretion of blood and protein in urine respectively), occur as a result of epithelial damage during the passage of eggs in the urogenital tract during *S. haematobium* infection, and are useful morbidity markers in SAC and in adults (Wilkins *et al.,* 1979, Feldmeier *et al.,* 1982, Mott *et al.,* 1983, Sousa-Figueiredo *et al.,* 2009, Webster *et al.,* 2009, King and Bertsch 2013, Ismail *et al.,* 2014, Ochodo *et al.,* 2015). They can be evaluated using inexpensive urine dipstick methods (microhaematuria) as well as visual colorimetry (visible haematuria) (Lengeler *et al.,* 2002, Bocanegra *et al.,* 2015). Their significance as POC field markers has been demonstrated in PSAC in Nigeria (Salawu

and Odaibo 2014) and Zimbabwe (Wami *et al.,* 2015). This is particularly useful as haematuria strongly correlates with infection intensity (Ismail *et al.,* 2014, Ochodo *et al.,* 2015) and has proven to be useful during post-treatment monitoring surveys, as morbidity reverses with treatment (King and Bertsch 2013). In addition, studies have shown that even in populations with the same infection intensity, the sensitivity and utility of microhaematuria decreases with age (Nduka *et al.,* 1995, Akogun and Obidiah 1996, Mafe 1997, Etard 2004a). Hence, microhaematuria is more likely to perform better as a morbidity marker in PSAC. A high urine albumin–creatinine ratio (UACR) translates to impaired kidney function due to increased albuminuria and decreased creatinine clearance (Snyder and Pendergraph 2005). UACR correlates with patent *S. haematobium* infections and urinary tract pathology in SAC (Rollinson *et al.,* 2005, Stothard *et al.,* 2009). In a recent study evaluating field markers of *S. haematobium*-related morbidity in Zimbabwean PSAC, UACR was found to be most reliable, and correlated with haematuria and proteinuria (Wami *et al.,* 2015). This suggests the significance of multiple field tools as morbidity indicators in PSAC, especially for *S. haematobium*.

Detection of soluble *Schistosoma* egg antigens and eosinophil cationic proteins (Kahama *et al.,* 1999, Reimert *et al.,* 2000) are more sensitive than indirect measures of morbidity (e.g. egg counts), as they detect early inflammatory reactions from *S. haematobium* infections in the urogenital tract (Reimert *et al.,* 1993). Thus, they can be useful field indictors of schistosome infection and morbidity, as seen in SAC (Reimert *et al.,* 2000). However, since disease sequelae may vary even in individuals with the same infection intensity (as detected by egg counts), elucidating their applicability in PSAC will be useful.

Faecal occult blood (FOB), the presence of cryptic blood in stool, results when *S. mansoni* eggs perforate the intestinal mucosa and cause a small release of blood into the bowel (Proietti and Antunes 1989, Betson *et al.,* 2010). Calprotectin, a multimeric complex of calcium-binding proteins MRP8/S100A8 and MRP14/S100A9, is secreted into the gut lumen when

calprotectin-containing granulocytes relocate into the intestinal mucosa in response to inflammation caused by schistosome eggs perforating the intestinal lining (Foell *et al.,* 2009). In this regard, the utility of FOB and calprotectin have been evaluated as morbidity indicators of intestinal schistosomiasis in PSAC in Uganda (Betson *et al.,* 2010, Betson *et al.,* 2012). In the aforementioned studies, FOB and calprotectin correlated positively with *S. mansoni* infection pre- and post-treatment.

Currently, there is a need to understand comorbidities due to different infections in PSAC, and how morbidity markers can be used to detect and determine the impact of schistosomiasis (Ouma *et al.,* 2001). The challenge with non-specific markers of morbidity is to determine how schistosomiasis influences them. However, this is a difficult task because even determining the fraction of morbidity attributable to schistosome infection does not indicate causation. Equally important is the lack of specific information on what they mean in terms of the child's overall current and future health. There is a need for a knowledge base addressing these two issues, if these morbidity markers are to form part of the diagnostic tool kit to inform the "diagnose and treat" approach for PSAC. The work presented in **Chapter 3** further reports findings on *S. haematobium*-related morbidity using morbidity markers including microhaematuria and malnutrition, and their corresponding morbidity attributable fractions to schistosome infection in PSAC.

1.11 Schistosome treatment in preschool-aged children

Praziquantel, discovered in 1979 (McMahon and Kolstrup 1979), is the antihelminthic drug of choice for treating schistosomiasis (World Health Organization 2020b). It comes in a 600 or 500 mg tablet used at a recommended dose of 40 or 60 mg/kg body weight. The lower dose is recommended for *S. haematobium* and *S. mansoni* infections while the higher dose (in two 30 mg/kg administrations) is recommended for *S. japonicum and S. mekongi* infections (WHO Expert Committee 2002). The safety and efficacy of PZQ, as measured by cure rate (CR) and egg reduction rate (ERR) has enabled its widespread use for schistosomiasis treatment. A

meta-analysis of studies showed that CR of between 63.5%–94.7% and ERR of 86.3%–95% for individual and mixed species infections, can be achieved with the recommended dose (Zwang and Olliaro 2014). Global and community-based schistosomiasis control programmes use PZQ through MDA for treatment, with an initial focus on morbidity reduction (World Health Organization 2020b). In resource-poor settings, a height–dose pole is used as a surrogate to weight scales for operational purposes (Hall *et al.,* 1999). Modifications have now been made to extend the original dose pole used for SAC and adults, to include height ranges for treatment of PSAC (Sousa-Figueiredo *et al.,* 2010, Sousa-Figueiredo *et al.,* 2012).

The current WHO recommended control strategy of PC with PZQ does not target PSAC (WHO Expert Committee 2002). It is recommended to treat PSAC with PZQ on a case-by-case basis, administered as crushed tablets with juice or bread (World Health Organization 2011, Bustinduy *et al.,* 2016a). This is due to the absence of a suitable paediatric formulation of PZQ for inclusion in MDA, as well as concerns over dosage and safety (Bustinduy *et al.,* 2016b); the drug was formally licensed for 4-year olds and above (Colley *et al.,* 2014, Bayer and Merck & Co 2020). There is increasing literature that the currently available PZQ is safe and efficacious in PSAC (Mutapi *et al.,* 2011, Coulibaly *et al.,* 2017, Montresor and Garba 2017, Coulibaly *et al.,* 2018). Recent randomised dose-ranging trials showed that a single 40 mg/kg dose of PZQ is effective for schistosomiasis treatment in PSAC (Coulibaly *et al.,* 2017, Coulibaly *et al.,* 2018). As described in previous sections of this chapter, a challenge to effective treatment in PSAC is the need for improved diagnostics. Other challenges that remain unique to PSAC include a drug that is more child-friendly, increased availability of treatment for PSAC in endemic areas, how to access PSAC for treatment, as well the ability to integrate treatment of PSAC into existing health systems (Stothard *et al.,* 2013, Bustinduy *et al.,* 2016a).

1.11.1 Praziquantel in preschool-aged children

Although PZQ is confirmed safe and efficacious, there is little information on its pharmacokinetics in PSAC (Cioli *et al.,* 2014, Olliaro *et al.,* 2014), and this is compounded by variable bioavailability, influenced by brand or co-administration with food (Mandour *et al.,* 1990, Dayan 2003). There is still a lack of knowledge surrounding PZQ's mechanism of action against schistosome adult worms. The mechanism of PZQ action thus far described, is that the drug affects parasite glutathione reductase and intracellular calcium levels, with secondary effects on parasite metabolism. The parasites die following disruption of calcium ion homeostasis, paralytic muscular contraction, and tegumental damage. This exposes the parasite antigens on the surface of the worm to attack by the immune system. The worms are then displaced from their site of action into systemic circulation or destroyed through phagocytosis (Dollery 1999, Doenhoff *et al.,* 2008, Thomas and Timson 2018). PZQ however lacks efficacy against immature juvenile schistosomes (Gönnert and Andrews 1977), which later mature, pair up and begin to deposit eggs; this contributes to the observed low cure rates and morbidity associated with the disease in certain instances (Ross *et al.,* 2015).

In humans, PZQ is nearly completely absorbed by passive diffusion after oral administration (Dinora *et al.,* 2005), with peak serum levels in 3–4 hours (Dayan 2003) and a half-life of 1.5 hours (Dollery 1999). Administration of PZQ with a carbohydrate meal enhances absorption and bioavailability of PZQ, including in PSAC (Mandour *et al.,* 1990, Castro *et al.,* 2000, Castro *et al.,* 2002). PSAC tolerate PZQ well with few reports of adverse effects, normally abdominal pain, vomiting, fatigue, and diarrhoea that resolve within 24 hours (Sousa-Figueiredo *et al.,* 2010, Mutapi *et al.,* 2011, Namwanje *et al.,* 2011, Coulibaly *et al.,* 2017, Coulibaly *et al.,* 2018). Contrary to suggestions of a higher efficacy dose in PSAC (i.e. >40 mg/kg) (Olliaro *et al.,* 2011), recent studies have reported on the efficacy and safety of escalating doses of PZQ in PSAC (i.e. 20 mg/kg, 40 mg/kg and 60 mg/kg) (Coulibaly *et al.,* 2017, Coulibaly *et al.,* 2018). PZQ showed a flat dose-dependent efficacy in PSAC, with the

maximum effect observed at the 40mg/kg dose; the same dose presently used (40 mg/kg) in SAC and adults therefore remains the best option in PSAC (Montresor and Garba 2017).

There are also limited human studies on the efficacy of PZQ administered along with other drugs or food. In PSAC living in helminth-endemic areas, PZQ is commonly administered with the antihelminthic drug albendazole as a deworming package, per WHO recommendations (World Health Organization 2006). A study published in 2011, reported that administering PZQ along with albendazole results in a greater combined effect of PZQ action, than PZQ alone (Lima *et al.,* 2011). Elucidating the effects PZQ may also have on the total exposure of albendazole when co-administered will be important to improve therapeutic strategies (Pawluk *et al.,* 2015). A recent study suggests that crushing of tablets and administration with food and drinks in PSAC also leads to pharmacokinetic differences when compared to adults (Bustinduy *et al.,* 2016b). Further studies elucidating the mechanisms behind such effects in PSAC will improve therapeutic efficacy.

As shown in experimental studies, PZQ is metabolised by the cytochrome P450 enzymes (Masimirembwa and Hasler 1994, Li *et al.,* 2003b). Thus, the growing evidence of genetic diversity for cytochrome P450 variants in schistosome-endemic regions in Africa (Rajman *et al.,* 2017) has implications for PZQ metabolism and treatment efficacy. With regards to PZQ metabolism and efficacy, PSAC should therefore be considered as a separate entity, especially in studies on treatment strategies and in the development of a paediatric PZQ formulation. This is because genetic variations, especially across African populations can influence the pharmacological effect of PZQ, and these must be taken into account (Buzkova et al., 2006). For instance, investigations into cases of PZQ resistance as observed in experimental and field studies, indicate that parasite sensitivity accounts for a limited proportion of reduced PZQ efficacy, and thus such inherent population characteristics are important (Cupit and Cunningham 2015, Vale et al., 2017).

1.11.2 Need for a paediatric praziquantel formulation

The WHO has recognised that PSAC in schistosome-endemic areas are at a high risk for infection, and that schistosomiasis presents a significant public health problem in this age group. It is recommended that treatment with the current PZQ tablets be administered through regular health services on a case-by-case basis, in the absence of an appropriate paediatric formulation (World Health Organization 2011). Despite the availability of a PZQ syrup that is equally efficient (Navaratnam *et al.,* 2012), limited availability, limited field utility, and high cost makes its use a challenge (World Health Organization 2011). Operationally, the large size and bitter taste of the existing PZQ tablet are associated with administration and compliance difficulties in PSAC (Stothard *et al.,* 2008, Sousa-Figueiredo *et al.,* 2010). The current tablet has to be broken into smaller pieces to obtain appropriate paediatric doses (based on weight), and is often crushed and administered with fruit juice or bread to make it palatable (World Health Organization 2011). These existing operational gaps make the use of the current tablets and treatment approach expensive and time-consuming (Navaratnam *et al.,* 2012). In addition, until recently when dose-ranging trials where conducted in PSAC (Coulibaly *et al.,* 2017, Coulibaly *et al.,* 2018), there had been concerns over knowledge gaps on dosage and safety, as the current 40mg/kg was an extrapolation of the SAC and adult dosing system (Bustinduy *et al.,* 2016b). Regulatory gaps still exist, as the current tablet was formally licensed for, and based on safety profiles, established in children 4 years old and above, as previously mentioned (Colley *et al.,* 2014, Bayer and Merck & Co 2020).

Based on existing operational, knowledge and regulatory gaps, and to ensure correct dosage and safe administration of treatment, a target product profile (TPP) has been recommended for the formulation of a flexible solid oral paediatric PZQ tablet (World Health Organization 2012b, Mduluza and Mutapi 2017). This TPP includes: (i) masked taste, (ii) oral dispersibility, (iii) fewer side effects, (iv) smaller size, and (v) environmental stability. The Paediatric Praziquantel Consortium, a public–private partnership, has produced a product meeting this

TPP, where the S–enantiomer (D-PZQ) that contributes to the bitter taste in the current tablet formulation has been removed, leaving the R-enantiomer (L-PZQ) associated with the antischistosomal properties of PZQ. A phase III clinical trial in children aged 3 months–6 years is currently ongoing in countries including the Ivory Coast and Kenya (Paediatric Praziquantel Consortium 2020). Longitudinal studies to determine the optimal timing and frequency of treatment in PSAC will be vital to inform deployment of this treatment. As detailed below, it will also be critical to investigate the best strategy to access PSAC for treatment.

1.12 Accessing preschool-aged children for schistosome treatment and control

In helminth-endemic areas, PC programmes targeting SAC are conducted via MDA with antihelminthics, usually PZQ and albendazole, administered in the school setting (WHO Expert Committee 2002, World Health Organization 2006). PSAC are not accessible in schools (although some may be in early child development centres), and the "diagnose and treat" recommendation, pending roll-out of the paediatric PZQ, makes it difficult to treat them outside a health setting. A retrospective study in Kenya found that four years of school-based MDA, resulted in a significant reduction in infection prevalence among school children but not at the population level (Lelo *et al.,* 2014). Thus, there is the need to upscale treatment strategies in order to render preventive measures more effective. It has been suggested that the increased treatment costs for accessing and treating PSAC will still render treatment programmes more cost-effective than current school-based strategies (Lo *et al.,* 2015).

One strategy is to access PSAC through the Expanded Programme on Immunization (EPI) in primary health centres (World Health Organization 2011). Studies in Zimbabwe have utilised the local primary health centres as a means to access PSAC, showing high compliance even in follow-up studies (Mutapi *et al.,* 2011, Mutsaka-Makuvaza *et al.,* 2018). In other countries, Child Health Days and Mother and Child Days offer a potential opportunity for easily reaching PSAC (World Health Organization 2011). The utility of such public health interventions has

been demonstrated in Uganda for albendazole treatments (Alderman *et al.,* 2006). However, in some health systems Child Health Days and EPI programmes may already be overwhelmed from implementing multiple interventions at the same time. Hence studies to monitor any changes or constraints of such integrated activities on existing health intervention programmes, as well as into alternative access strategies are required. Recently, an approach of empowering health workers to identify clinical cases (i.e. non-specific signs of suspected cases), making PZQ available in health centres, and integrating the paediatric PZQ into immunization programmes have also been suggested (Bustinduy *et al.,* 2016a). Some of the strategies outlined above can also be crucial for including PSAC in monitoring and evaluation of treatment across endemic countries, for more accurate national and regional schistosomiasis metrics (Faust *et al.,* 2020). Longitudinal studies are needed to ascertain the best approach and timing suitable to access PSAC for chemotherapy across a diversity of endemicities.

1.13 Complementary schistosome control methods

Modelling studies have postulated that treatment strategies alone, without other interventions such as snail control and improved sanitation conditions will not be sufficient to achieve sustainable schistosomiasis control and elimination (Gurarie *et al.,* 2015, Secor and Colley 2018). Fundamental facets that are key to ensuring reduced schistosome transmission, and ultimately, elimination in endemic countries include improved water, sanitation and hygiene (WASH), behavioural change, targeted education, and snail control (Sokolow *et al.,* 2016).

A review of evidence shows that WASH interventions designed to prevent contact with infective water sources and reduce transmission are likely to reduce the odds of schistosome infection (Grimes *et al.,* 2015). With regards to PSAC, an overall safe environment can be provided through provision of latrines, alternative water sources, and education on good hygiene practices for older individuals and caregivers, all of which indirectly promote efforts at reducing contact and infection in PSAC. Education of the caregiver (through interactive materials) can change customs and behaviours that put PSAC at risk of infection, and also

encourage carer participation in control programmes with their young ones (WHO Expert Committee 2002, Beanland *et al.,* 2006). Snail control measures include chemical methods such as molluscicide (Yuan *et al.,* 2011), and biological methods such as the introduction of biological competitors like *Tilapia melanopluera* (Stauffer *et al.,* 2006) and river prawns (Sokolow *et al.,* 2015). However, chemical methods as used in parts of China can be expensive and time-consuming, especially for large areas, and can be toxic to other aquatic life (Yang *et al.,* 2010). Despite the challenges, snail control measures target all age groups at risk of infection, by interrupting the complex life cycle of schistosomes. This is important to reduce the number of water-borne infectious cercariae, and ultimately the risk of (re)infection. Environmental control methods including vegetation and silt removal from water bodies and the use of improved farming methods (e.g. improved excreta storage), have also proved useful in agricultural settings (Jordan *et al.,* 1993).

Praziquantel treatment alone will not be sufficient for sustainable schistosomiasis control and elimination (Secor and Colley 2018). Integrated schistosomiasis control that includes improved treatment coverage combined with the complementary methods outlined above, has been shown to be more cost-effective over time (Lo *et al.,* 2018).

1.14 Outlook

There is now a global move for increasing research on paediatric schistosomiasis and promoting control of the disease. This is not only to benefit child health but also to move towards the goal of eliminating schistosomiasis. The clinical definition of schistosome pathology and morbidity in PSAC remains to be improved and refined. Better diagnostic tools are key for schistosomiasis control in PSAC. High hopes rest on the results of the Phase III clinical trials of the leading formulation of paediatric PZQ from the Paediatric Praziquantel Consortium. Studies on strategies to access PSAC for treatment as well as to determine the optimal time to treat, will inform its deployment. This chapter of my thesis brings into context important current knowledge and gaps in paediatric schistosomiasis research and practice, and

how the work in subsequent chapters contribute to the current body of knowledge. In the next sections, I describe the specific aims and rationale, along with the outline of this thesis.

1.15 Thesis rationale

Over the last decade the WHO's goal has been to reduce schistosomiasis morbidity by 2020, and eliminate schistosomiasis as a public health burden by the year 2025 (World Health Organization 2012a). It appears this milestone will be missed. As we enter the new decade, the WHO has published an updated neglected tropical disease (NTD) roadmap for 2021–2030 (World Health Organization 2020a). The updated roadmap indicates that despite recent efforts, critical action is required for expanding research and treatment to all populations, especially PSAC, and for sustainable control and elimination of schistosomiasis. In schistosome-endemic areas, children carry most of the infection burden, and at least 25 million of these are PSAC (WHO Expert Committee 2002, World Health Organization 2011). Despite evidence of schistosome infection and the recommendation to treat PSAC with schistosomiasis (World Health Organization 2011), this age group is still excluded from treatment programmes in most endemic areas. The consequences of such inequity in health are that while PSAC remain significant reservoirs for infection transmission and hamper elimination efforts (Lelo *et al.,* 2014, Njenga *et al.,* 2014), the intensity of infection continues to accumulate until about age 6 years, when they receive their first treatment through school-based MDA. Some of the knowledge gaps for excluding PSAC from treatment include a poor understanding of schistosome exposure, infection, disease dynamics and treatment, and a lack of a coherent strategy to access and treat PSAC. Operational difficulties in the diagnosis of infection, as well as the perspective of a low infection risk (Sacko *et al.,* 2011) and lack of a known immune response to infection or response to treatment have also been contributing factors (Mutapi 2015a).

Currently, the global infection and disease burden is not fully known in this age group; the best estimates available on the burden of paediatric schistosomiasis are from published data

from scientific studies, focused on describing the prevalence of infection and morbidity in endemic areas (Woolhouse *et al.,* 2000, Bosompem *et al.,* 2004, Odogwu *et al.,* 2006, Mutapi *et al.,* 2011, Kemal *et al.,* 2019, Mutsaka-Makuvaza *et al.,* 2019, Sacolo-Gwebu *et al.,* 2019). Longitudinal studies to describe the incidence of infection and morbidity, especially the early events that occur during the very first infection and treatment in PSAC are lacking. In addition, parasite-related growth and nutritional disorders are significant childhood problems in schistosome-endemic areas (Freer *et al.,* 2018). However, there are no studies detailing the attributable fraction of malnutrition due to schistosomiasis, the impact of treatment on these factors, and their utility to identify high-risk groups in endemic areas. These knowledge gaps make it difficult to make operational and economic plans for controlling schistosomiasis in this age group.

With the current recommendation to treat PSAC on a case-by-case basis and pending the roll out of the paediatric PZQ, longitudinal studies to determine different treatment timings and strategies for maximum health benefits of treatment in terms of reduced reinfection and morbidity rates are required. This is based on the evidence that as shown in older children and adults, PZQ treatment in PSAC also induces schistosome-specific immune responses associated with resistance to reinfection (Rujeni *et al.,* 2013). This will be important to determine strategies and opportunities for early screening and treatment of PSAC as part of routine health interventions, while reducing transmission and reinfection, and preventing irreversible pathology in PSAC.

While there is an opportunity to influence the health of PSAC through specifically designed interventions such as nutraceuticals, there are limited human studies on the mechanistic pathways of disease and treatment in young children. Much of our understanding of the host-parasite interactions that contribute to parasite survival and disease development are from experimental studies. There is a need for mechanistic studies that contribute more to

understanding the molecular and biochemical mechanisms underlying schistosome-related morbidity and treatment in PSAC.

1.16 Thesis aims

The specific aims of this thesis were as follows:

(i) To determine using currently available field tools, the prevalence of *S. haematobium* infection and morbidity in PSAC, and the proportion of growth and nutritional sequelae associated with schistosome infection. The incidence of the first *S. haematobium* infection and morbidity, and the impact of treatment on infection and reversal of early morbidity was also determined.

(ii) To determine the operational health benefits of regular quarterly screening and treatment of schistosome infections (upon first infection), in terms of reducing subsequent new infections and reinfections in PSAC. The reinfection rates and intensity observed following a single PZQ treatment of the first schistosome infection in PSAC, was compared to that observed with the treatment of chronic infections.

(iii) To characterise the host metabolite profiles of PSAC before their first *S. haematobium* infection, and the changes that occur following infection and curative treatment. The impact of specific metabolite alterations on host metabolic pathways and schistosome-related morbidity was also determined.

(iv) To characterise the structure (abundance and diversity) of the gut microbiome and resistome in PSAC, and to determine the association between *S. haematobium* infection and the gut microbiome and resistome.

1.17 Thesis outline

The study presented in this thesis explored the dynamics and consequences of *S. haematobium* infection and disease in PSAC. In this chapter, the current knowledge and gaps in paediatric schistosomiasis practice, and how the work presented in this thesis contributes to this knowledge base are described.

Chapter 2 outlines the study design related to the specific aspects of this study, as well as methodological considerations related to field work and laboratory analysis. The main statistical methods used to analyse the data described in this thesis are also outlined.

In contributing to data on the infection and disease burden in PSAC, **Chapter 3** focuses on the utility of currently available tools to determine the incidence of the first *S. haematobium* infection and morbidity, how quickly disease develops post-infection, as well as the impact of treatment on infection and reversal of early morbidity in PSAC. It further describes the proportion of growth and nutritional sequelae observed in PSAC, that is attributable to schistosome infection.

Chapter 4 examines the operational health benefits of regular quarterly screening and treatment of *S. haematobium* infections (upon first infection), in terms of reducing subsequent new infections and reinfections in PSAC. The health benefits (in terms reinfection rates and intensity) of a single PZQ treatment of the first *S. haematobium* infection in PSAC, versus that observed with the treatment of chronic long-standing infections was also examined.

Chapter 5 describes the impact of *S. haematobium* infection and treatment on the host metabolic phenotype, as well as the impact of infection on metabolism and morbidity in PSAC.

Chapter 6 characterises the structure of the gut microbiome and resistome of PSAC. It further describes the influence of *S. haematobium* infection on the abundance and structure of the gut microbiome and resistome, as well as its impacts on host health in PSAC.

Chapter 7 summarises and discusses the major findings presented in this thesis in broader terms. It further describes the contribution of my findings to paediatric schistosomiasis practice and health policy, and provides suggestions for further research.

Chapter 2 Methods

2.1 Introduction

Decades of studying host-pathogen interactions to understand infection and disease mechanisms relating to the aetiology, as well as clinical manifestations of morbidity and immunopathology, have relied heavily on experimental models of infection (Sarkar and Heise 2019). In the case of schistosomiasis, studies in animal models have provided insights into infection and disease mechanisms. However, the paradigms presented by experimental schistosome studies are not easy to extrapolate to humans due to differences in the natural history of infection (Woolhouse 1998, Woolhouse *et al.,* 2000). For example, while a single exposure to cercariae can result in infection in mouse models, human infection in endemic areas is more likely due to repeated exposure (Cheever *et al.,* 2002). In addition, T-helper type 1 (Th1) immune responses have been associated with protection from infection in mouse models, while predominantly T-helper type 2 (Th2) immune responses have been associated with protection in humans (Hagan *et al.,* 1991, Hagan 1993, Mutapi *et al.,* 1998, Mutapi *et al.,* 2006, Tebeje *et al.,* 2016). Such marked differences and their relevance to the complex human disease forms and associated heterogeneities, highlight the need for more human studies.

The challenges of conducting infection and mechanistic studies in humans have limited our understanding about the pathophysiology of the disease in humans, as illustrated by urogenital schistosomiasis (Rollinson 2009). The variations in susceptibility to infection, disease manifestation, genetics, underlying health issues, persistent coinfections, and demographic factors such as age and gender have limited human infection studies (Sarkar and Heise 2019). While human challenge infections are possible, limitations for such studies to be conducted in a controlled environment, with controlled infection doses or route of infection, and with self-limiting disease (Selgelid and Jamrozik 2018), makes it difficult to follow and fully understand the natural time-course of infection and disease in humans. Ethical limitations also make it difficult to perform mechanistic studies in humans (Darton *et al.,* 2015). It was only recently

that a controlled *Schistosoma mansoni* infection human model was developed (Langenberg *et al.,* 2020). Although this is promising and paves the way for rapid proof-of-concept mechanistic, vaccine and drug studies, the infection dynamics and immune responses clearly differed from that observed in a natural course infection in endemic settings. For instance, infections were single-sex, and did not produce eggs that contribute to the chronic immunomodulatory mechanisms observed in natural infections (Langenberg *et al.,* 2020).

Thus, the work presented in this thesis was based on following natural schistosome infections (focusing on *S. haematobium*) in preschool-aged children (PSAC), aged 5 years and below, living in schistosome-endemic areas in Zimbabwe. The field study involved a baseline cross-sectional survey followed by a longitudinal infection–treatment–reinfection study design, focusing on various aspects of infection, disease and treatment dynamics. Overall, the thesis involved quantitative analysis of epidemiological factors associated with *S. haematobium* infection and morbidity measured in the field, in combination with laboratory analysis of biological samples.

For field studies, methods including the currently recommended parasitological detection of parasite eggs in urine or stool (World Health Organization 2020b), and the detection of haematuria as a measure of morbidity for *S. haematobium* (Lengeler *et al.,* 2002, Bocanegra *et al.,* 2015) were used. Despite the operational challenges of sampling and diagnostic sensitivity of current conventional methods (Bergquist *et al.,* 2009, Coulibaly *et al.,* 2013, Wami *et al.,* 2014, Utzinger *et al.,* 2015), there is lack of a more convenient, specific, rapid, and cheap alternative diagnostic suitable for field applications, especially for *S. haematobium*. Although immunological methods can overcome the issues of reduced sensitivity with parasitological diagnosis (e.g. in detecting low intensity infections, prepatent or single sex infections), issues of cost, cross-reactivity and difficulty in blood sampling of PSAC pose challenges in this case (Kanamura *et al.,* 1998, Oliveira *et al.,* 2005). In addition, the more sensitive point-of-care (POC)–CCA test to detect worm antigens in urine has also shown to be

a reliable tool for the diagnosis of *S. mansoni* infections (Coulibaly *et al.,* 2013, Ochodo *et al.,* 2015), but less so for *S. haematobium* infections, the focus of this thesis (Ashton *et al.,* 2011, Sousa *et al.,* 2019). The use of parasitological methods to describe the dynamics of infection and treatment in this thesis enables comparison of findings to other studies, and to inform developing targeted interventions, while parasitological methods remain the predominant schistosome diagnostic in PSAC in schistosome-endemic areas. *Schistosoma* -omics research continues to contribute significantly to the understanding of disease dynamics and in the exploration of innovative diagnostics and treatment (Wang and Hu 2014). The integration of such methods into routine readouts in helminth studies has been recommended (Kokova and Mayboroda 2019). Thus, the work presented in this thesis also employed the methods of genomics and metabolomics, using a combination of DNA sequencing, spectroscopic methods, multivariate pattern recognition methods, and bioinformatic techniques. The use of such analytical techniques while controlling for confounding effects, is effective at overcoming the limitations of heterogeneity in human infection studies (Wang *et al.,* 2004, Balog *et al.,* 2011). These were used to analyse the overall changes in the metabolome and gut microbiome in response to schistosome infection and treatment, and how these relate to mechanisms of disease progression and morbidity in PSAC. Overall, the findings from this thesis, based on the methods used, contribute to gaps relating to infection, disease and treatment dynamics, as well as their implication on new ways of reducing current and future burden of schistosomiasis in PSAC.

In this chapter, I describe the main study design on which the work from this thesis was based, the study participants and site, the field and laboratory methods used, as well as the statistical methods applied to answer specific research questions in this thesis. Specific details relating to the methods outlined here are in the relevant chapters as appropriate.

2.2 Field study

2.2.1 Ethical approval and consent

The study received institutional approval from the University of Edinburgh (fmutapi-0002) and ethical approval from the Medical Research Council of Zimbabwe (MRCZ/A/1964). Permission to conduct the study in the province was obtained from the Provincial Medical Director. Prior to enrolment, the overall and specific aims and procedures associated with the study were explained to all participants' parents/guardians in their local language, Shona. Written informed consent was obtained from the participants' parents/guardians as appropriate. Recruitment into the study was voluntary and parents/guardians were free to withdraw participants at any time with no further obligation.

2.2.2 Study population and site

The study was conducted in four villages, Chihuri, Mupfure, Madziwa, and Nyamaropa, located in the Shamva district, Northeast of Zimbabwe (17°10′0″S 31°40′0″E) (see **Figure 2.1**). This is one of seven districts in the Mashonaland Central province of Zimbabwe, which has about 123,650 people living in a predominantly rural area of 2,695 km², according to the 2012 national census (Zimbabwe National Statistics Agency 2012). The area was selected for this study on urogenital schistosomiasis because the prevalence of *S. haematobium* is high (>50% endemicity), while the prevalence of *S. mansoni* and soil-transmitted helminths (STH) is low (<15%) (Midzi *et al.,* 2011, Midzi *et al.,* 2014b). The inhabitants from the study villages are of similar ethnicity (Shona) and socioeconomic background (primarily subsistence farmers). There is poor sanitation provision and inadequate safe water sources (as indicated by study questionnaires and observations from the study site). Residents use nearby rivers for farming, domestic water supply, and recreational activities. Activities in the area confirm that exposure to schistosome infection in older children is active through direct water contact activities, while a significant amount of the exposure in PSAC is passive through activities of their caregivers, and becomes more active with age (Odogwu *et al.,* 2006, Garba *et al.,* 2010).

PSAC aged 5 years and below were recruited for the study. Recruitment was based in centres used by the community for the Expanded Program for Immunisation (EPI), i.e. crèches, early child development centres, preschools and primary health centres. Parents/guardians of children not in any educational programmes (e.g. children <3 years old) were invited through the village health workers to report to the sampling centres with their children for enrolment into the study.

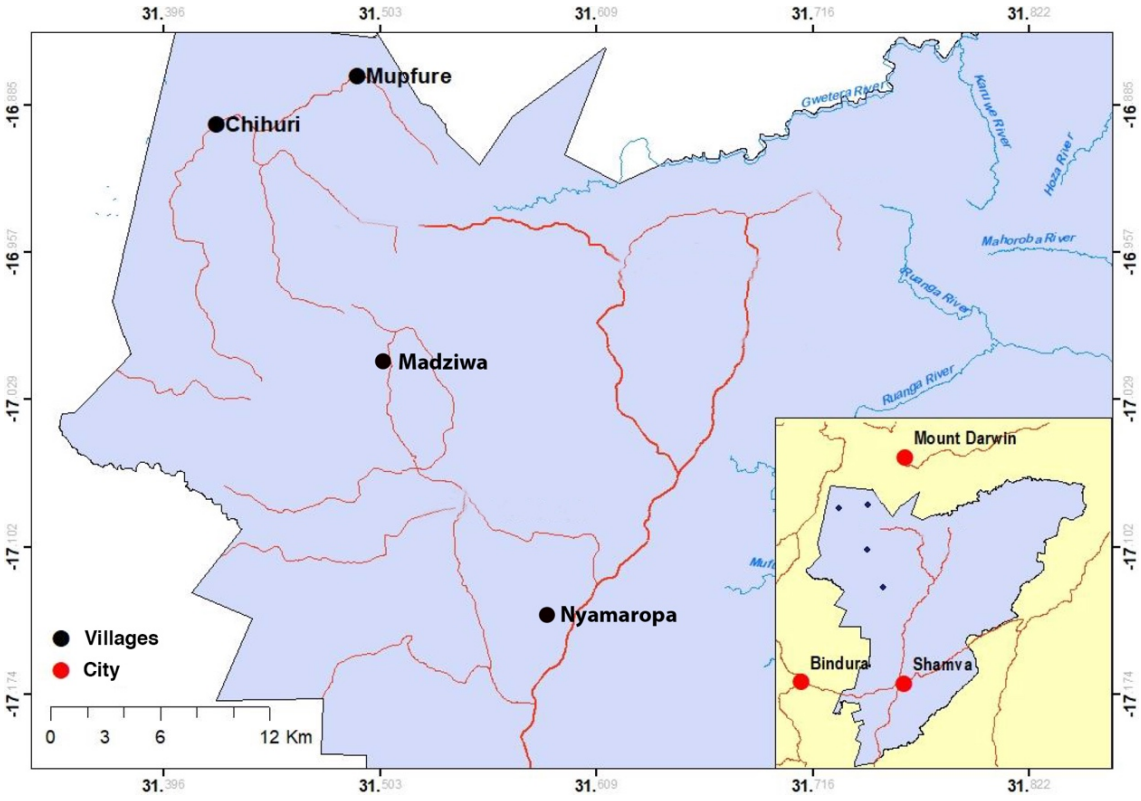

Figure 2.1: Map of study area showing locations of the four villages where participants were enrolled
Map was produced using the software package ArcMap 10.1.

2.2.3 Study design

The work presented in this thesis was based on data obtained from field studies as part of a larger project, "Childhood Schistosomiasis: A Novel Strategy Extending the Benefits/Reach of Antihelminthic Treatment", in collaboration with the University of Edinburgh and the University of Zimbabwe. The study was conducted from February 2016 to February 2018, and

included a baseline cross-sectional study, followed by a standard longitudinal infection–treatment–reinfection study design. The study was designed to detect the difference in reinfection and schistosome-related morbidity rates between two groups of previously screened and treated PSAC (using quarterly versus annual regimes). There were two main aspects of the longitudinal study: (i) to determine and treat new schistosome infections using two different strategies, quarterly versus annual screen and treatment (year 1), and (ii) to determine the impact of quarterly versus annual screen and treatment in terms of reinfection and morbidity rates, at least 12 months post-treatment (year 2). A schematic diagram of the study design is shown in **Figure 2.2**.

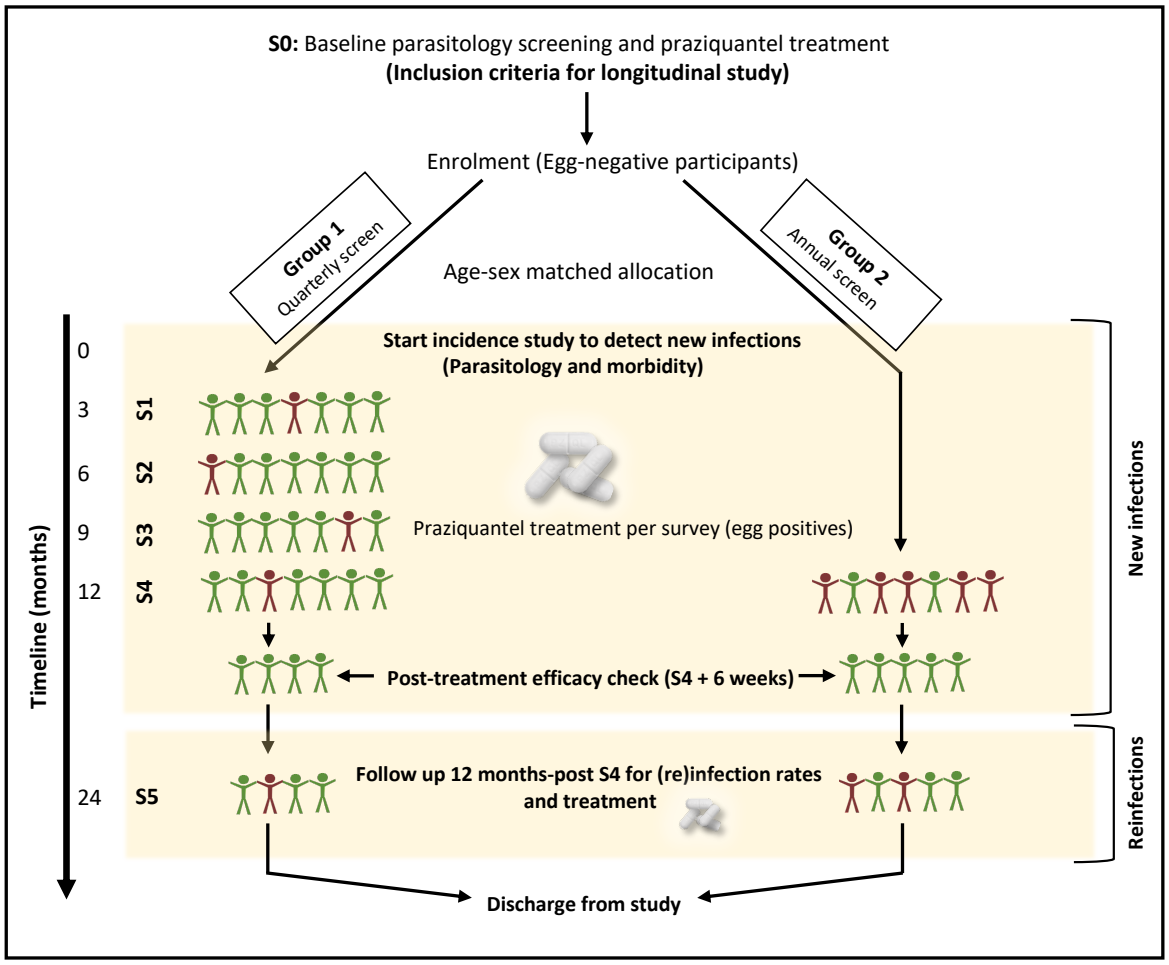

Figure 2.2: Schematic diagram showing the larger field study design and survey follow-up times
Post-baseline cross-sectional survey (S0), age- and sex-matched *S. haematobium*-negative children were randomly allocated to one of two groups. Group 1 was followed up every 3 months in surveys S1, S2, S3, S4, and at 12 months in survey S4 for Group 2, for parasitology and morbidity**.** Both groups were followed up at 24 (S5) months for parasitology and morbidity. Red cartoon, *S. haematobium* positive; Green cartoon, *S. haematobium* negative

At baseline (survey S0), a cross-sectional study to diagnose *S. haematobium* infection and morbidity (measured by microhaematuria), and to exclude *S. mansoni* and other intestinal helminth infections was carried out. Children positive for schistosome infection by egg count were treated with praziquantel (PZQ) and excluded from further follow-up. Children confirmed egg-negative were age- and sex-matched, and randomly allocated into one of two groups, Group 1 and Group 2. Children in Group 1 were screened at 3, 6, 9, and 12 months (surveys S1–S4) for detection of infection and morbidity within 3 months of first infection.

The 3-month screening interval takes into account the 10–12 week period for new *S. haematobium* infections to reach patency and be detectable by egg excretion (Webbe and James 1971, Smith *et al.,* 1976, Wami *et al.,* 2016). Children in Group 2 were screened and treated once at 12 months (survey S4). The 12-month period for Group 2 was chosen to represent the current practise of annual mass drug administration (MDA) in Zimbabwe (targeted at SAC and adults).

PZQ treatment was offered to children found to be positive for infection at each survey. A post-treatment efficacy check was carried out for all treated children at each subsequent follow-up survey, i.e. 3 months post-previous survey for Group 1 (survey S1–S3), and 6 weeks post-S4 survey for both groups). To assess the impact of the different screening and treatment strategies on reinfection and morbidity, children in both groups were followed up at 24 months (survey S5) for reinfection and morbidity.

2.2.4 Sample size calculation

The sample size was calculated to address the primary aim of the larger project, which was to compare reinfection rates following different treatment regimens, i.e. between children receiving early screening and treatment upon first detection of infection (Group 1), versus children screened and treated annually (Group 2). Calculations were based on assumptions that early treatment of infection (Group 1) will reduce the reinfection rate by at least half (50%), in comparison with the current standard annual treatment method (Group 2). Based on the reinfection rates observed in children after 12 months from an earlier study conducted by our research group (Parasite Immuno-epidemiology Group) (Mutapi *et al.,* 2011, Midzi *et al.,* 2014a), this corresponds to 15% in Group 1 versus an expected 30% in Group 2. Statistical power analysis was performed using Gpower version 3.1.5 (Faul *et al.,* 2007). Based on an expected prevalence of 6.7%, as derived from our research group's previous study in 1–5 year olds (Wami *et al.,* 2016), and given $\alpha = 0.05$, and power = 0.80, the sample size required given the effect size was 214 in each group, following the baseline survey. Allowing for a 20%

dropout rate, the minimum sample size required for each group during follow-up was 256 in each group. In order to obtain the number of infected children sufficient to detect the reinfection rates hypothesised, it was necessary to start with larger sample sizes of uninfected children for each group, by including all children found eligible for the longitudinal study, following the baseline survey.

2.2.5 Eligibility criteria

At baseline, the study included PSAC (≤5 years old) who met the following inclusion criteria: (i) consent from parents/guardians, (ii) they were lifelong/permanent residents of the study area, (iii) they had no history of antihelminthic treatment (assessed by clinical records and questionnaires administered to parents/guardians for all children), (iv) they were exposed to infective water (as established by initial water contact questionnaire and malacology survey of the water contact sites), and (v) they had provided at least two urine samples for parasitological diagnosis of *S. haematobium*, and at least one stool sample for parasitological diagnosis of *S. mansoni*/STH on consecutive days, and (vi) they were negative for *S. mansoni* and STH (Hookworms, *Ascaris lumbricoides* or *Trichuris trichiura*). Children were excluded if they had pre-existing medical conditions, presented with clinical symptoms of tuberculosis, had malaria/fever or showed signs of being unhealthy, or had a recent major illness/surgery (assessed upon examination by field study clinicians, clinical records and reports from parents/guardians).

To be included in the longitudinal cohort, children who had fulfilled the inclusion criteria described above had to meet an additional criterion of being diagnosed negative for *S. haematobium* infection by egg count at baseline, to track the incidence of first new infections.

2.2.6 Questionnaires

A questionnaire, designed in English and translated into the local language (Shona), was administered by the research team to gather demographic data and establish exposure behaviour. Parents or guardians responded to the questionnaires on behalf of their children, in addition to validation from clinical records and child health cards. Prior to start of the study, the questionnaire was pretested in a sample of the population to ensure the questions and answers reflected accurate information as sought by the questionnaire. A copy of the questionnaire can be found in **Appendix D**.

2.2.7 Anthropometry

Anthropometric measurements were conducted by the research team, and for very young children, measurements were taken by local nurses from the Madziwa rural clinic (see **Figure 2.3**). Weight (nearest 0.1 kg) and height (nearest 0.1 cm) without shoes and in light clothing was measured using an electronic scale (Seca 878dr, Seca GmbH & Co. KG., Birmingham, UK) and a stadiometer (Seca 217, Seca GmbH & Co. KG.) respectively. For very young children, height was measured with an infantometer baby board (Seca 416, Seca GmbH & Co. KG.), and weight measured with a baby weighing scale (Seca 384, Seca GmbH & Co. KG.). The mid-upper arm circumference (MUAC) was measured (nearest 1 mm) using a child MUAC tape (Ibis Medical Equipment & Systems Pvt. Ltd., India). This was measured on the left arm, midpoint between the shoulder and the tip of the elbow, with the arm relaxed and hanging down the body.

Figure 2.3: Panel showing images from anthropometric measurements in the field
Image shows measurements for weight, height, and Mid-upper arm circumference (MUAC) as part of anthropometric measurements.

2.2.8 Parasitological diagnosis

Sample processing for parasitological tests were performed by the research team, and microscopy was done by trained field technicians from the University of Zimbabwe. Approximately 50 ml of at least two separate urine samples were collected from each participant on three successive days, and a stool specimen was collected on a single day from each participant. The collection of replicate samples on different days was done to improve sensitivity of diagnosis due to variation in egg excretion (Engels *et al.,* 1996). Samples were collected between 10:00 hours and 14:00 hours and processed within two hours of collection. For very young children, urine bags (Hollister 7511 U-Bag Urine Specimen Collector, Hollister Inc., Chicago, IL, USA) and disposable diapers were used to collect urine and stool samples respectively.

Urine samples were examined microscopically for *S. haematobium* infection, following the standard urine filtration method (Mott *et al.,* 1982). In summary, each urine sample was thoroughly mixed, and 10 ml of urine was aspirated using a 10 ml plastic syringe. A

nitrocellulose mesh filter was then attached, and the urine slowly passed through. If present, parasite eggs were left trapped in the filter, which was then observed under a light microscope and number of eggs/10 ml of urine enumerated. Stool samples collected were processed using the Kato–Katz thick smear method (Katz *et al.,* 1972). Stool samples were sieved to remove large particles, and duplicate slides were prepared using the standard 41.7 mg templates and stained with glycerol–malachite green (stains stool components but does not penetrate parasite eggs) to aid parasite egg identification. Parasite eggs of intestinal helminths were enumerated under a light microscope per gram of stool (41.7 mg multiplied by 24), as per World Health Organisation (WHO) guidelines (World Health Organization 1994). Children were diagnosed positive for helminth infection if at least one parasite egg was detected in their urine or stool samples. *S. haematobium* infection intensity was expressed as the arithmetic mean egg count/10 ml of at least two urine samples, collected on three consecutive days. A panel of images from sample processing and microscopy for parasitological diagnosis is shown in **Figure 2.4**.

Figure 2.4: Panel showing images from processing of urine and stool samples for parasitology in the field

2.2.9 Diagnosis of morbidity

Schistosoma haematobium-related morbidity, measured as the presence of blood in urine, was detected using a POC visual colorimetry and dipstick urinalysis method (**Figure 2.5)**. Urine samples collected were first examined for macrohaematuria (visible haematuria) and recorded as positive or negative based on presence or absence of visible blood in urine respectively. Where quantities of blood in urine was too low to change the colour of urine and be detected visually, urine was assayed for microhaematuria using reagent strips (Uristix®, Uripath, Plasmatec, UK). The reagent end of the strip was dipped into fresh, well-mixed urine for about 40 seconds, and the test area was compared to a standard colour chart as per manufacturer's

instructions. Where no blood was present in urine, there was no colour change, and this was recorded as Negative. The strength of colour change on the strip, indicating varying concentrations of blood present in the sample was reported as Trace, Positive (+), Positive (++), Positive (+++), or Positive (++++). For analysis purposes as used in the work presented in this thesis, any colour change indicating microhaematuria (including Trace) was coded as Positive.

Figure 2.5: Panel showing images from morbidity detection, measured by haematuria in the field

2.2.10 Praziquantel treatment

At all survey time points, PZQ was administered to children positive for schistosome infection at the recommended single standard oral dose of 40mg/kg, in tablet form (Cipla Ltd, India, manufactured for MedPharm) (World Health Organization 2011). Treatment was carried out and supervised by local nurses from the Madziwa rural clinic. Treatment was administered as crushed tablets, given with juice and bread to reduce the bitter taste and side effects of PZQ as recommended by the WHO (Mutapi *et al.,* 2011, World Health Organization 2011). A post-

treatment efficacy check (via egg count) was carried out for all previously treated participants, at each subsequent follow up, and participants who were found to be egg-positive were treated with a repeat 40 mg/kg dose of PZQ. Reports of adverse side-effects from PZQ treatment in PSAC are few, and limited to abdominal pain, vomiting, fatigue, and diarrhoea that resolve within 24 hours (Sousa-Figueiredo *et al.,* 2010, Mutapi *et al.,* 2011, Namwanje *et al.,* 2011, Coulibaly *et al.,* 2017, Coulibaly *et al.,* 2018). All treated children were observed for an hour by field clinicians for immediate adverse side-effects, and to determine if treatment was lost through vomiting. Children were monitored again by field clinicians the following day (with the caregiver or parent present) to report any side-effects that were absent the day before.

2.2.11 Stool DNA isolation and storage

Aliquots of fresh stool samples were taken and processed for microbiome studies. Stool samples in 2 ml cryotubes were stored temporarily at 2–8 ºC for a maximum of 24 hours prior to processing. Studies have shown that storage of fresh stool samples from 24–72 hours at room temperature or at 4 ºC for up to 48 hours, does not significantly alter the observed microbiota sequences, when compared to freshly processed samples or samples rapidly stored at -20 ºC or -80 ºC (Wu *et al.,* 2010a, Tedjo *et al.,* 2015). All samples were preserved in the same manner to reduce inter-sample variation (Carruthers *et al.,* 2019).

DNA was extracted from stool using the QIAamp DNA Stool Mini Kit (QIAGEN, catalogue number 51504), according to manufacturer's instructions. This method of DNA extraction from stool has been shown to be appropriate for microbiome studies (Li *et al.,* 2003a). In brief, stool samples were homogenized in buffer and heated at 95 °C to lyse cells. An InhibitEx tablet was added to remove potential inhibitors, and the lysates were treated with proteinase-K and buffer at 70 ºC for 10 minutes to remove protein and polysaccharides. Isolated DNA was then precipitated by ethanol, applied to a column and washed twice with buffers. The DNA was eluted and then dissolved in buffer. DNA was stored at -20 ºC in the field and

transferred to a -80 ºC freezer in the laboratory at the University of Zimbabwe until shipped on ice to the University of Edinburgh for long-term storage at -80 ºC.

2.2.12 Blood sampling, processing and storage

Collection of blood samples were carried out by local nurses from the Madziwa rural clinic. Up to 5 ml of venous blood was collected from each participant into serum separator tubes (BD Vacutainer®) and processed for serum for serological and biochemical assays. To minimise the effects of temporal and mealtime variability, especially on biochemical assays (serum metabolite analysis), non-fasting pre-meal samples were taken at about midday during field surveys (Kim *et al.,* 2014). Blood samples were allowed to clot at room temperature for about 30 minutes, and were stored at 4 °C for a maximum of 4 hours prior to processing. Samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. Aliquots of serum samples were frozen at -20 °C in the field and transferred to a -80 °C freezer in the laboratory at the University of Zimbabwe until shipped on ice to the University of Edinburgh for long-term storage at -80 ºC.

2.2.13 Field data handling and cleaning

In terms of field work, I was involved in each of the research methods and procedures described above, to obtain the data required for this study. All de-identified field data was entered in a Microsoft Excel spreadsheet, and to ensure accuracy, I facilitated a double entry verification. Prior to any statistical analyses, data was cleaned and verified to correct for any inconsistencies. Data cleaning and verification involved comparing spreadsheet data to all hard copy raw data entered from the field.

2.3 Metabolomics methods

2.3.1 Sample preparation

Approximately 50 µl each of serum samples were shipped on dry ice to Human Metabolome Technologies Inc. (HMT; Yamagata, Japan) for sample preparation and metabolite analysis. At HMT, each 50 µl sample was mixed with 450 µl of methanol containing HMT in-house internal standards (10 µM). Chloroform (500 µl) and Milli-Q water (200 µl) were then added, mixed thoroughly and centrifuged (2,300 x g, at 4°C for 5min). The water layer (400 µl) was passed through a filter (ULTRAFREE-MC-PLHCC; HMT, Yamagata, Japan) to remove macromolecules, with a 5-kDa cut-off. The filtrate was centrifugally concentrated and resuspended in 50 µl of ultrapure water immediately before the measurement.

2.3.2 Metabolite measurement

Compounds were measured in the cation and anion modes of capillary electrophoresis time-of-flight mass spectrometry (CE–TOF–MS)-based metabolome analysis (Soga and Heiger 2000, Soga *et al.,* 2002, Soga *et al.,* 2003). To improve the quality of the capillary electrophoresis mass spectrometry (CE–MS) analysis, samples were diluted 1:2 and 1:5 for cation and anion modes respectively.

Cationic metabolites (i.e. in cation mode) were analysed with a fused silica capillary (i.d. 50 µm × 80 cm), using Cation Buffer Solution (p/n: H3301-1001; HMT) as both the run and rinse buffer. Sample injection was done at a pressure of 50 mbar for 10 seconds at an applied capillary electrophoresis (CE) voltage of 27 kV. Electrospray ionization mass spectrometry (ESI–MS) was conducted in the positive ion mode with a capillary voltage of 4,000 V. Mass spectrometer (MS) scanning range was a mass-to-charge ratio (m/z) of 50–1,000 using an HMT in-house sheath liquid (p/n : H3301-1020). All other conditions were as used in cation analysis mass spectrometry (Soga and Heiger 2000). Anionic metabolites (i.e. in anion mode) were analysed with a fused silica capillary (i.d. 50 µm × 80 cm), using Anion Buffer Solution (p/n: H3302-1021; HMT) as both the run and rinse buffer. Sample

injection was done at a pressure of 50 mbar for 25 seconds at an applied CE voltage of 30 kV. ESI-MS was conducted in the negative ion mode with a capillary voltage of 3,500 V. MS scanning range was a m/z of 50–1,000 using an HMT in-house sheath liquid (p/n: H3301-1020). All other conditions were as used in anion analysis mass spectrometry (Soga *et al.,* 2002). A schematic diagram of the metabolite measurement workflow is shown in **Figure 2.6**. Details of instrumentation are described in the following section.

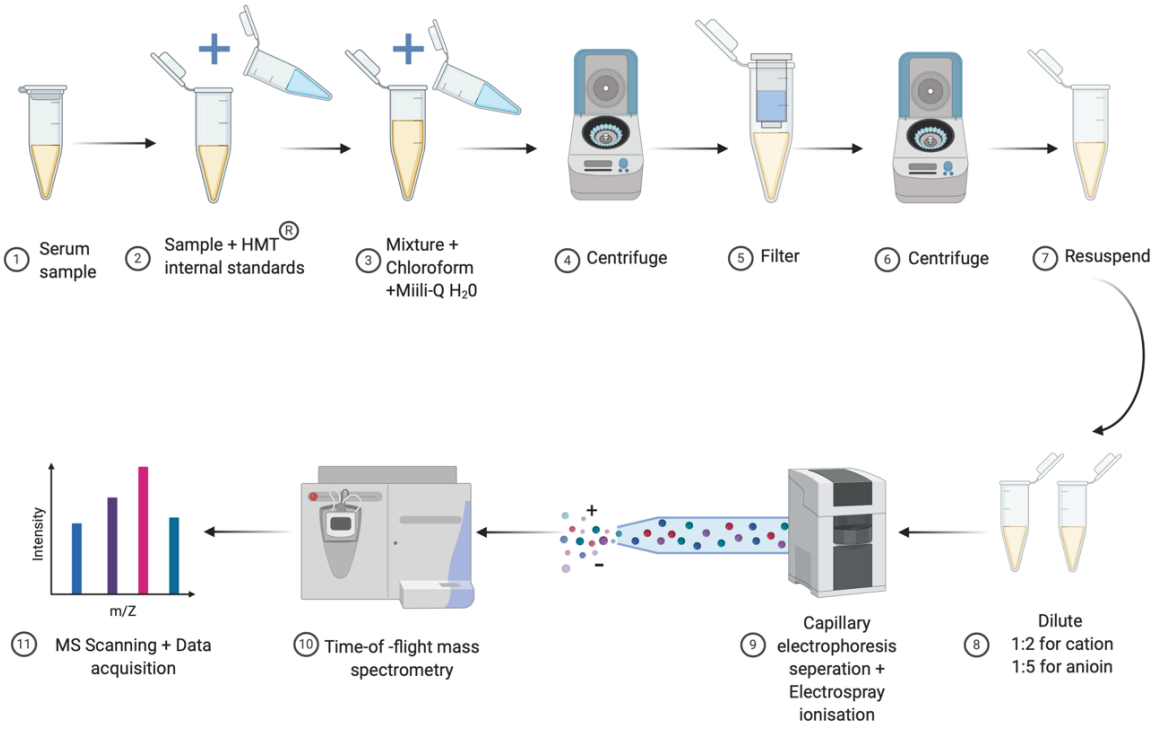

Figure 2.6: Schematic diagram of the serum metabolite mass spectrometry process

2.3.3 Instrumentation

CE–TOF–MS was carried out using an Agilent CE–TOF–MS system (Agilent Technologies Inc. Waldbronn, Germany), equipped with an Agilent 6210 time-of-flight mass spectrometer (TOF–MS), Agilent 1100 isocratic high-performance liquid chromatography (HPLC) pump, Agilent G1603A CE–MS adapter kit, and Agilent G1607A capillary electrophoresis electrospray ionization mass spectrometry (CE–ESI–MS) sprayer kit (all from Agilent

Technologies, Waldbronn, Germany). System control, data acquisition and evaluation were facilitated by Agilent G2201AA ChemStation software version B.03.01 for CE (Agilent Technologies). To facilitate thermostating of the capillary, the CE–MS adapter kit includes a capillary cassette. The CE–ESI–MS sprayer kit simplifies coupling the CE system with mass spectrometry systems equipped with an electrospray source. The sprayer is designed to give an orthogonal flow in order to reduce the detrimental effects caused by the charged particles or droplets (Voyksner and Lee 1999).

2.4 Microbiome studies

2.4.1 Sequence library preparation and sequencing

DNA samples were shipped on dry ice for library preparation and sequencing at the Beijing Genomics Institute (BGI, Shenzhen, China). To ensure sample aliquots contained adequate amounts of purified DNA, each sample was quantified in-house at the University of Edinburgh using the Qubit fluorometer (ThermoFisher Scientific), prior to shipment for library preparation and DNA sequencing. At BGI, DNA was quantified using the Qubit fluorometer (ThermoFisher Scientific) and NanoDrop^{TM} (ThermoFisher Scientific). The integrity and purity of DNA was then assessed by a 1% agarose gel electrophoresis, run at 150V for 40 minutes. DNA passing quality control was sheared by ultrasonication into fragments (Covaris S/E210). Fragments were mixed with End Repair Mix (BGI), adaptors were ligated to the ends of the DNA fragments, and then purified using the QIAquick Polymerase Chain Reaction (PCR) Purification Kit (QIAGEN). Adapter-ligated DNA fragments were separated by electrophoresis through a 2% agarose gel to recover the target fragments and purified using the QIAquick Gel Extraction kit (QIAGEN). Library preparation to enrich the adapter-ligated DNA was done via PCR amplification, size-separated by electrophoresis, and then purified using the QIAquick Gel Extraction Kit (QIAGEN). The final library was quantified using the Agilent 2100 bioanalyzer. The qualifying DNA libraries were amplified using the cBOT system (Illumina), and sequenced on the Illumina Hiseq 4000 platform (Illumina) using

paired-end 150-bp sequencing. Raw sequence data was received as FASTQ files for further processing and analysis (see **Figure 2.7**).

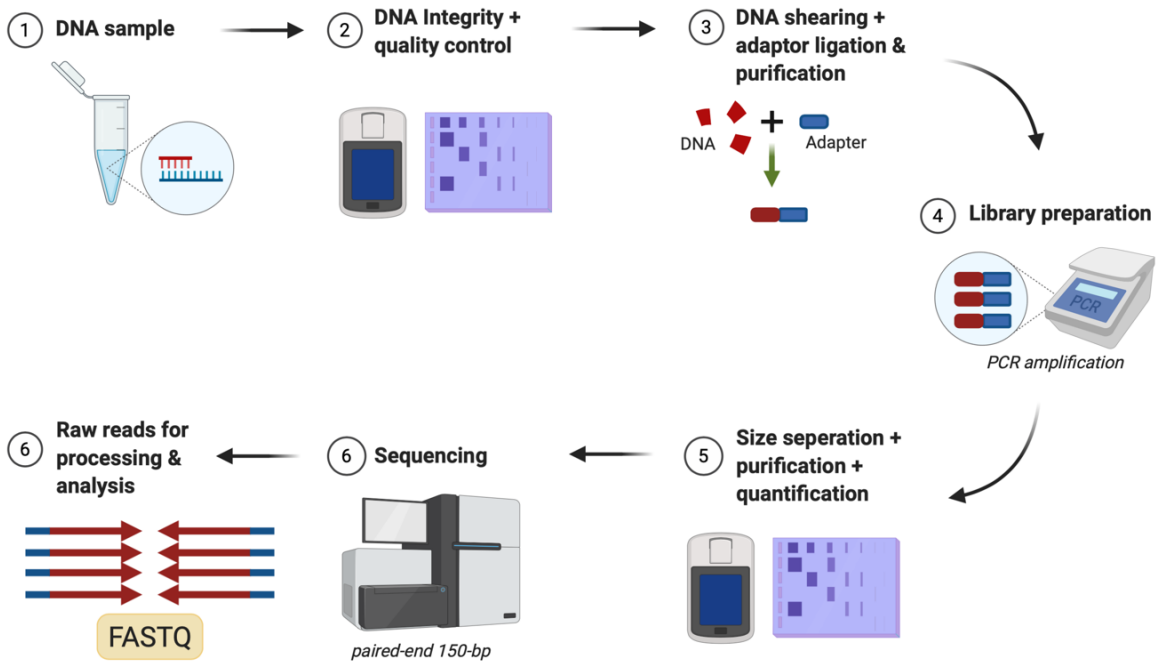

Figure 2.7: Schematic diagram of the DNA sequencing process

2.4.2 Mapping of sequence reads

Direct mapping of sequence reads against reference sequence databases was done using a novel reference-based mapping and alignment tool known as k-mer alignment (KMA) (Clausen *et al.,* 2018). This method was used because it is designed to improve mapping against redundant databases, and has been shown to outperform existing mapping methods, in terms of speed, precision and sensitivity (Clausen *et al.,* 2018). In summary, KMA, employs heuristic mapping, which involves mapping k-mers between query sequences and selected template databases, including large redundant databases, then assigns a score to matching k-mers. K-mers with positive scores are then used as seeds from which to proceed for alignment; each matching seed is extended and given an optimal score and mismatch regions are identified. KMA then utilizes a special version of the Needleman-Wunsch algorithm

(Needleman and Wunsch 1970) to accurately align regions of mismatching k-mers. To ensure the best match template for the query reads, multi-mapping reads are then resolved using a novel sorting scheme known as ConClave. The scheme takes all initially accepted best matching templates and sums up the alignment scores for each template to obtain the ConClave score, from which the best matching sequence template is chosen. A unique ConClave score is computed for uniquely mapped reads, and for multi-mapped reads which may have the same ConClave score, the algorithm favours the first template added to the database. Although in such scenarios both templates will be equally suited candidates (and likely be variants of the same template), this is to ensure that by consistently favouring the parent template added to the database, there is reproducibility.

The above processes enable assembly of reads which results in a final consensus sequence for the reference or template sequence. The strength of each called nucleotide is tested for overrepresentation using the McNemar test ($\alpha = 0.05$), to rule out bias associated with base calling across different sequencing platforms (Kaas *et al.,* 2014). A schematic diagram of the process is shown in **Figure 2.8**.

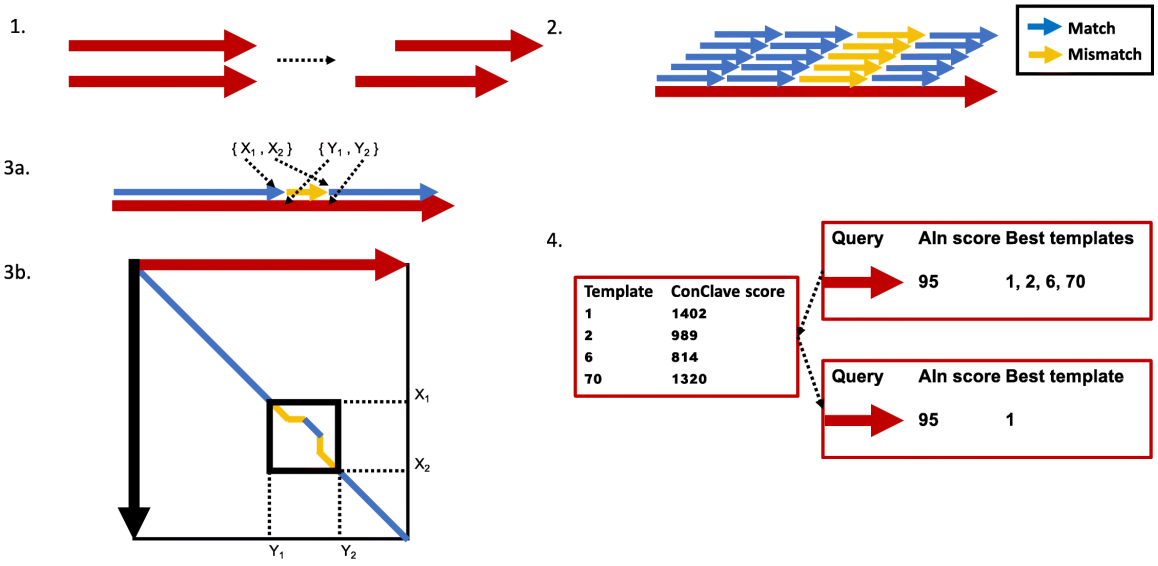

Figure 2.8: Overview of the k-mer alignment (KMA) algorithm
Trims are read (1), then k-mers matched between query and database (2). Matching k-mer seeds are extended, regions with mismatching k-mers are identified (3a) and aligned using the Needleman-Wunsch algorithm (3b). A Conclave scoring is then used to choose one best-aligning template per query sequence (4). Image adapted from (Clausen *et al.,* 2018).

2.5 Statistics and data visualisation

Data were analysed using SPSS version 22 (IBM Corp.), and with the R software (www.bioconductor.org; www.r-project.org) (R Development Core Team 2011), unless otherwise stated. Data visualization was performed with R and with GraphPad Prism version 7.02 or 8.2.0 (GraphPad Software, Inc.). Here, I present an overview of the main statistical methods used to summarise and test the different hypotheses used in this thesis. Specific details on where these were used to answer each research question are in the relevant chapters as appropriate.

2.5.1 Univariate analyses

For all analyses, data were first checked for normality, i.e. if they assumed a normal distribution. In addition to frequency distribution plots, the D'Agostino-Pearson normality test (omnibus K2 test) was used to ascertain normality of data (D'Agostino and Stephens 1986). The method first computes the skewness (measure of how symmetrical the data is) and kurtosis

(the degree of tailedness of the data relative to a normal distribution), compares these to the expected values of a normal Gaussian distribution, and computes a p-value from the sum of any discrepancies found. Where data was normally distributed, parametric statistics were used. In cases where data were aggregated or skewed, as with schistosome infection intensity, which is typically heavy in only a small proportion of people (Gryseels *et al.,* 2006), data were transformed to meet normality assumptions and parametric statistics used.

2.5.1.1 Parametric tests

Parametric techniques used for data analyses include the following:

(i) Descriptive statistics were used for exploratory data analyses, and to provide summaries on measures of central tendencies (i.e. mean), along with measures of dispersion including the range, standard deviation (SD), standard errors (SE). For example, as used in this thesis to determine the mean schistosome infection intensity.

(ii) The Pearson's correlation coefficient (r) was used to measure the strength and direction of linear relationships or associations between pairs of continuous variables or within and between sets of continuous variables. For example, as used in this thesis to determine the relationship between serum metabolite concentrations and schistosome infection intensity.

(iii) A fold change (FC) analysis was used to compare absolute value changes between two group means, such as to determine the mean fold changes in metabolite concentrations between groups.

(iv) The student's t-test was used to compare means between two independent groups or where appropriate, a paired t-test, to compare two means from the same group at different time points. For example, the t-test was used in this thesis to determine the difference in mean schistosome infection intensity between groups.

(v) A general linear model (GLM) was used to investigate the relationship between variables of interests to predict an outcome, and where appropriate, accounted for potential confounders. The method is flexible in that it allows the use of predictor (explanatory) variables measured on different scales (continuous and categorical), as was the case with the work presented in this thesis. This included the analysis of variance (ANOVA) and linear regression models, consisting of both categorical and continuous variables, e.g. to determine the influence of schistosome infection intensity and microbiota abundance. GLM assumes certain conditions; residuals (difference between predicted and actual values) are normally distributed, there is homogeneity of variance of residuals, the individual variables under consideration are independent, and an assumed linear relationship between the dependent and independent variables.

2.5.1.2 Non-parametric tests

Where normality assumptions were not met, or in cases where discrete or categorical data were involved, non-parametric statistics were used.

(i) Descriptive statistics were used for exploratory data analyses, and to provide summaries on measures of central tendencies (i.e. median), along with measures of dispersion including the range and interquartile ranges (IQR), e.g. the median age of the study population. Categorical data were summarised as absolute numbers and percentages, e.g. proportion of schistosome positive and negative individuals.

(ii) The Chi-square ($\chi 2$) test was used to determine associations between independent categorical variables, and where appropriate, the McNemar's statistic for paired categorical data, e.g. as used to determine the difference in the schistosome infection rates observed at baseline and at follow up, compared between different

groups (independent) or within the same group (paired). Where small sample sizes were involved, the Fisher's exact test was used.

(iii) The generalised linear model was used to model categorical response or dependent variables against independent variables. This is an extension of the GLM, which takes into account instances where response variable distributions did not meet normality assumptions. This included the multiple logistic regression, e.g. as used to determine the influence of schistosome infection status on the presence or absence of stunting (adjusted for age and sex), as well as the age-dependent modelling approach which was used to determine the predicted age–infection prevalence relationship, as proposed by Diamond and McDonald (1991) and Keiding (1991).

2.5.2 Multivariate analyses

Where analysis of data involved more than one dependent or response variable which were related or correlated with each other or had to be considered together (e.g. in this thesis, serum metabolite profiles), multivariate analyses were used. Below, I outline details of the multivariate analysis methods used in this thesis.

2.5.2.1 Principal Component Analysis

Principal Component Analysis (PCA) was used to provide an informative first-hand look at datasets, and to assess group clustering and separation trends. This method involves using data reduction techniques to summarise underlying variabilities in multiple dependent variables, through linear combinations of these variables (principal components), each of which explains a percentage variation (Le Cao *et al.,* 2016). For example, this was used to uncover existing trends, clusters or outliers in the data set of microbiota abundance between schistosome-infected versus uninfected children. Generally, the first component has the most variability, whiles the subsequent components are a linear combination of the maximum variance that is uncorrelated with the previous components.

2.5.2.2 Orthogonal Projections to Latent Structures Discriminant Analysis

To determine variations in multiple dependent or response variables, relative to predicting differences between groups (e.g. metabolite signatures associated with schistosome infection), the Orthogonal Projections to Latent Structures Discriminant Analysis (OPLSDA) was used (Wiklund *et al.,* 2008). This is a supervised method that is more reliable at overcoming the limitations of heterogeneity associated with analysis of data from human studies, where PCA does not reveal separation trends (Singer *et al.,* 2007, Balog *et al.,* 2011). For instance, OPLSDA was used to reveal discriminatory metabolite profiles between schistosome-infected and uninfected groups.

OPLSDA is a modified version of the Partial Least Squares Discriminant Analysis (PLSDA) (Szymanska *et al.,* 2012), and has the capability to distinguish between variations in a dataset both relevant and irrelevant to predicting groups, while incorporating an Orthogonal Signal Correction (OSC) filtering (Wold *et al.,* 1998) into a partial least squares model. The model relates multiple response variables (Y) and "X" blocks of matrices (groups) by a linear multivariate model. It then separates the systematic variation between groups into two predictive (covariance between X and Y; between group variation) and orthogonal (systematic variation in X that is unrelated to Y; within group variation) components, free of interfering structured variation. Model statistics, R^2 and Q^2, were calculated for each model and used to assess the degree of fit and predictive reliability of the OPLSDA model respectively (Wold *et al.,* 2001). R^2 represents the fraction of the variance explained by a component in the model and is expressed as $R^2 = (1-RSS/SS)$, where RSS and SS are the fitted residual sum of squares and the sum of squares of the response variables respectively. The Q^2 is the cross-validated R^2, expressed as (1-PRESS/SS), where PRESS is prediction error of the sum of squares (Wold *et al.,* 2001).

A permutation testing that assumes there is no difference between any two groups compared was used to cross-validate and ensure the model was reliable and not over-fitted (Szymanska

et al., 2012). In summary, sample groups were randomly permuted, and a new classification model calculated. Model performance was then assessed by the R^2 and Q^2 diagnostic statistics, expected to be lower than that obtained for the original unpermuted dataset. The permutations were repeated 1000 times and the diagnostic statistics obtained were used to create a null distribution, H_0, of models expected to be non-significant. The diagnostic statistics for the OPLSDA model from the original data set was related to that of the H_0 distribution from the permuted data sets to determine the statistical significance (p-value) of the OPLSDA model:

$$p = \frac{1 + \#(Q^2 p \geq Q^2)}{N} \tag{2.1}$$

Where N is the number of permutations, $\#(Q^2p \geq Q^2)$ is the number of elements in the null distribution H_0, which are greater or equal to the Q^2 for the original data set (or otherwise R^2).

2.5.2.3 (PER)MANOVA

To determine if the mean differences between groups on the combination of multiple dependent or response variables is likely due to chance, a multivariate analysis of variance (MANOVA) was used. This is an extension of the ANOVA but incorporates multiple dependent or response variables (Weinfurt 1995), e.g. as used in this thesis to test the influence of schistosome infection status (adjusting for age and sex) on the combination of several host metabolite profiles (dependent variables). MANOVA creates a linear combination of each of the dependent variables (e.g. metabolite concentrations), which is then used to perform an ANOVA using the independent variables. The test assumes a normal distribution of the dependent variables, linearity between each pair of dependent variables, and homogeneity of variances and covariances (intercorrelations) of the dependent variables across the independent variables. Data was transformed to meet normality assumptions, and scatter plots were used to assess linearity between dependent variables.

In instances where the dependent or response variables were treated as dissimilarity measures (e.g. Euclidean distances, for abundance of microbiota), the permutational multivariate analysis of variance (PERMANOVA) was used. This was used to determine if there was any variation across a space of a multivariate dissimilarity measure, in response to one or more independent variables, while making no distributional assumptions (Anderson 2014). For instance, as used in this thesis to determine the variation in microbiota abundance with age, sex, village, and schistosome infection status.

2.5.2.4 ANCOM

To compare two or more populations accounting for the underlying compositional structure in a dataset (e.g. comparing two or more populations of microbial taxa), relative to two or more groups (independent variable, e.g. schistosome-infected versus uninfected groups), the analysis of composition of microbiomes (ANCOM) was used (Mandal *et al.,* 2015). This was more appropriate in this case than standard statistical methods for comparison because, the relative abundance of microbes within a specimen (in this case stool samples) sums up to one (1), resulting in compositional data and not one in a Euclidean space. Like PERMANOVA, ANCOM makes no distributional assumptions and uses bootstrapped intervals to perform hypotheses tests, while generating a W-value denoting a count of the number of times the null hypothesis (e.g. there is no difference in abundance of taxa between two groups) is rejected for a particular taxon. By definition, the W-value (the number of times the null hypothesis is rejected for a given taxonomic group) is the ratio of a specific taxonomic group and a number of other groups (i.e. the W value) that are significantly different across two groups. In all analyses, a specific taxon was considered significant when it varied across the independent variable of interest with respect to 80% of the rest of the taxa in the dataset (W-statistic cut off: 0.80).

2.5.3 General considerations

Statistical models included where appropriate, all biologically meaningful interactions between the independent variables considered, and all insignificant interactions were excluded from the final model. For instance, the MANOVA model to determine the effects of age, and sex on host metabolite profiles included age and sex interaction as a variable.

To control for multiple significance testing and to reduce the likelihood of type 1 error (false positives or the probability of rejecting the null hypothesis when it is true) the false discovery rate (FDR) approach was used (Benjamini and Hochberg 1995), unless otherwise stated. For example, the model on the effects of multiple metadata factors on microbiota taxa abundance was adjusted for multiple testing with an FDR threshold of 0.05.

For all analyses and hypothesis testing, a p-value of <0.05 was considered significant.

2.6 Estimating measures of growth and nutrition

Based on anthropometric measures, growth and nutritional status were assessed using the WHO Anthro software, version 3.0.1 (http://www.who.int/childgrowth/en/) (Mondal *et al.,* 2012). This is a tool developed by the WHO, to facilitate nutritional surveys and to promote best practices and a standardised approach to data analyses and reporting of anthropometric indicators in children 5 years old and below. The tool, based on raw anthropometric and demographic data (including age and sex), generates anthropometric estimates based on four indicators; length/height–for–age, weight–for–age, weight–for–length/height, and body mass index (BMI)–for–age. The output was a set of generated Z-scores for the specific measures from which growth and nutritional status was measured; stunting determined by height–for–age Z-scores (HAZ), underweight by weight–for–age Z-scores (WAZ) and BMI–for–age Z-scores (BAZ), as well as malnutrition by MUAC Z-scores (MUAC and MUACZ) and weight–for–height Z-scores (WHZ). Measures with Z-scores <-2 were considered abnormal (MOH Malawi 2016).

Chapter 3 Dynamics of *Schistosoma haematobium* infection and morbidity in preschool-aged children aged 6 months–5 years

Part of this work has been published (Osakunor *et al.,* 2018a) and a copy of the publication is included in **Appendix E.**

3.1 Introduction

In schistosome-endemic areas, exposure to schistosome infection is cumulative and increases as children grow. Almost all children will have been exposed to schistosome cercariae by age one, and the first infection typically occurs within the first 5 years of life (Woolhouse *et al.,* 2000, Wami *et al.,* 2014). Epidemiological studies show evidence of schistosome infection in preschool-aged children (PSAC; 5 years old and below) as early as when they are a year old (Woolhouse *et al.,* 2000, Bosompem *et al.,* 2004, Odogwu *et al.,* 2006, Mutapi *et al.,* 2011, Kemal *et al.,* 2019, Mutsaka-Makuvaza *et al.,* 2019, Sacolo-Gwebu *et al.,* 2019). Left untreated, infection can lead to significant morbidity including poor growth and cognitive development, chronic diarrhoea, and anaemia due to progressive blood losses (van der Werf *et al.,* 2003, Freer *et al.,* 2018). However, this age group has historically been considered to be at low risk of infection (Sacko *et al.,* 2011), with several studies quantifying infection and morbidity in school-aged children (SAC), i.e. ≥6 years and adults (van der Werf *et al.,* 2003, Gryseels *et al.,* 2006), but not enough comparable studies in PSAC. Consequently, the schistosome infection and morbidity burden in this age group, especially the earliest events that occur during the first infection, are not fully known and is likely underestimated, with negative impacts on on-going schistosome control programmes (Garba *et al.,* 2010).

Schistosome pathology and morbidity are still being defined in older children and adults in order to reflect manifestations of the disease, for example, as with female genital schistosomiasis (Kjetland *et al.,* 2012). In PSAC, pathology and morbidity are also still being defined in order to identify applicable morbidity markers of disease, for example, urine

albumin–creatinine ratio (UACR) (Wami *et al.,* 2015). In addition, growth and nutrition-related morbidities associated with schistosomiasis only recently became more widely recognized (King and Dangerfield-Cha 2008). Thus, in schistosome-endemic areas where coinfections and comorbidities are common, elucidating how much of these growth-related morbidities are due to schistosomiasis is of importance. There is therefore a need to address these gaps and to collate new knowledge to better define schistosomiasis in PSAC, where manifestations of disease are poorly described.

Recently, the World Health Organization (WHO) made a recommendation for the treatment of PSAC with schistosomiasis (World Health Organization 2011). The lack of a paediatric formulation of the antihelminthic drug, praziquantel (PZQ), has led to the exclusion of PSAC from mass drug administration programmes in endemic countries (Stothard *et al.,* 2011, Stothard *et al.,* 2013). Concerted efforts are beginning to address this, while PSAC are treated upon diagnosis, on a case-by-case basis (Bustinduy *et al.,* 2016a). However, in schistosome-endemic areas where the first infection typically occurs in PSAC, there is lack of adequate knowledge on the earliest events that occur during the first infection in this age group. Questions that remain unanswered include: When does infection occur and when can it be detected? What are the early morbidity indicators of infection? What are the implications of infection in PSAC on current and future health? Longitudinal studies to quantify exposure patterns with age, to track the incidence of new schistosome infection and morbidity, and to determine implications on current and future health of PSAC are also lacking.

To address these, I aimed to describe the dynamics of schistosome infection and morbidity in Zimbabwean PSAC exposed to *Schistosoma haematobium*, using existing field diagnostic and morbidity tools. I determined the prevalence of *S. haematobium* infection and morbidity in PSAC, and the proportion of growth and nutritional sequelae associated with the infection. Following a cohort of schistosome-negative children (not previously infected or treated) for a year, I determined the incidence of their first *S. haematobium* infection and morbidity, as well

as the impact of curative treatment on infection and morbidity. The findings of this chapter demonstrate the ability of existing diagnostic and morbidity tools to quantify and monitor early infection and morbidity in PSAC. It also contributes to addressing the knowledge gaps in the global infection and disease burden estimates, and the dynamics of infection and morbidity in PSAC. Consequently, this will inform the planning and implementation of control programmes targeted at this age group.

3.2 Hypotheses

(i) In schistosome-endemic areas, the first schistosome infection in PSAC can be detected early using the current predominant parasitological diagnostic methods.

(ii) The first infection with *S. haematobium* is associated with significant morbidity early in infection, and resolves following curative treatment with PZQ.

(iii) The chronic growth and nutritional disorders observed in PSAC in schistosome-endemic areas are attributable to schistosome infection.

3.3 Methods

3.3.1 Study design, population and site

This was a longitudinal study embedded within the larger paediatric urogenital schistosomiasis study, including PSAC aged 6 months to 5 years who met the inclusion criteria for recruitment at baseline, as previously described in **Chapter 2**. For the current study, a cross-sectional study was conducted at baseline, followed by a one-year longitudinal study. To be included in the longitudinal cohort, children must have fulfilled an additional criterion of being diagnosed negative for *S. haematobium* infection by egg count, as part of the baseline survey. A schematic diagram of the study design is shown in **Figure 3.1**.

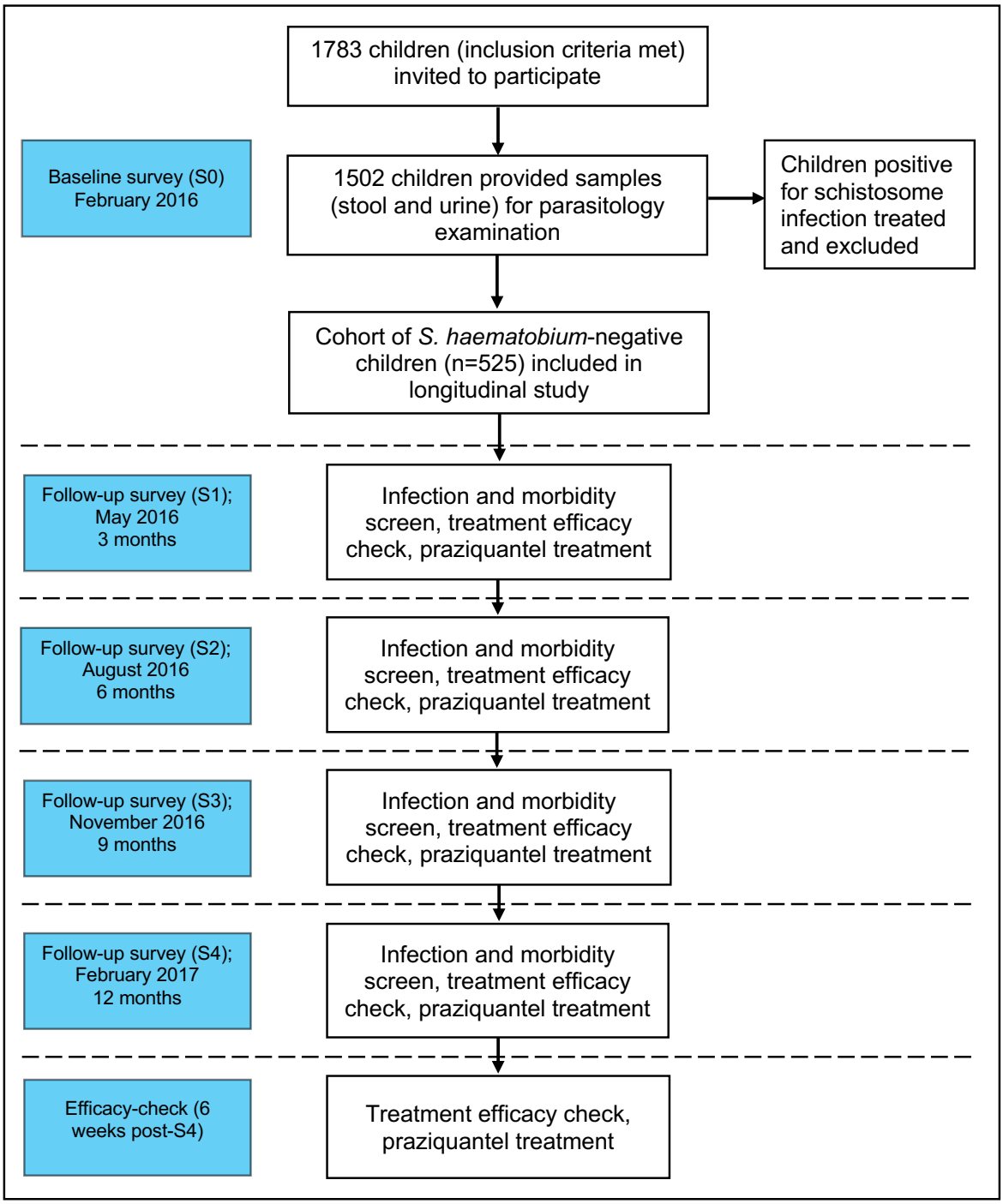

Figure 3.1: Flow chart of study design

At baseline, recruited children were screened for schistosome infection and morbidity, to describe infection and morbidity prevalence. Metadata on socio-demography (including age and sex) and anthropometry (including weight, height and mid-upper arm circumference

(MUAC) gathered from questionnaires administered at the time of recruitment were used (described in **Chapter 2**). Of the children who were recruited and invited to participate at baseline (n=1783), 1502 children provided samples for parasitological diagnosis of schistosome infection. Following this, a group of children confirmed *S. haematobium* negative (n=525) and had not been previously infected or treated for schistosomiasis (assessed by health questionnaire and clinical records, as described in **Chapter 2**), were invited to form the longitudinal cohort. This group of children were followed up every quarter to detect their first schistosome infection by egg count, and morbidity by microhaematuria. The 3-month follow-up period was chosen because experimental and previous field studies show that in 3 months, new *S. haematobium* infections can reach patency and thus be detectable by egg excretion (Webbe and James 1971, Smith *et al.,* 1976, Wami *et al.,* 2016). At each survey time point, all children who were positive for schistosome infection were treated with a single dose of PZQ at the standard 40 mg/kg body weight, as described in **Chapter 2**. A post-treatment efficacy check by parasitology was carried out for all previously treated children at each subsequent follow-up survey (3 months after each previous survey for S1–S3, and at 6 weeks post-treatment for the last survey at S4).

3.3.2 Sample collection and processing

Urine and stool samples were collected for parasitological diagnosis of *S. haematobium* infection (all survey time points) and to exclude *S. mansoni* and soil-transmitted helminths (baseline only) respectively. *Schistosoma haematobium*-related morbidity was measured as the presence of macrohaematuria and microhaematuria, using a point-of-care visual colorimetry and dipstick urinalysis respectively. Details are as previously described in **Chapter 2**.

3.3.3 Data handling and statistical analyses

Growth and nutritional indices adjusted for age and expressed as Z-scores (Mondal *et al.,* 2012) were calculated using the WHO Anthro software (version 3.0.1), as previously

described in **Chapter 2**. Measures with Z-scores <-2 were considered abnormal (MOH Malawi 2016). Data analyses were performed using SPSS version 22 (IBM Corp.), and graphs were prepared using GraphPad Prism version 7.02 (GraphPad Software, Inc). Infection intensity was expressed as the arithmetic mean egg count/10 ml of at least two urine samples collected on three consecutive days. To meet the normality assumption of parametric statistics, infection intensity data was log-transformed ($\log_{10}$ [egg count + 1]). The Chi-square test (or Fisher's exact test for small sample sizes) and the Mann-Whitney test were used to test for differences in categorical and continuous variables respectively.

To determine the relationship between age and schistosome infection intensity, and to predict how infection intensity changes with age, a linear regression model was used. Age (years) was used as the explanatory variable and infection intensity as the response variable. To model the infection prevalence (based on a binary response; positive or negative for infection) as a function of age (explanatory), the age-dependent logistic regression model was used (Diamond and McDonald 1991, Keiding 1991). The prevalence was then fitted as the probability of being infected at a specific age, *a*, using the prevalence probabilities generated from the model.

To determine the effect of multiple factors on the prevalence of schistosome infection and morbidity status (i.e. positive or negative for both indices), multiple logistic regression was used. This was done to determine the likelihood of presenting with schistosome infection and morbidity, based on multiple factors, and the results were reported as adjusted odds ratios (AOR) and 95% confidence intervals (CI), along with the test for significance. Models were built using the forward stepwise inclusion of the main terms and their interactions. This method was preferred because: (i) the main effects model was used as the baseline model, with no potential for inflated mean squared errors (average squared difference between the predicted and actual values), and thus a more accurate model, and (ii) the model can be easily assessed to avoid saturation with too many terms, which would compromise the consistency and efficiency of model parameter estimations (Gould and Lawless 1988). Two-way

interactions were included in all models, but none was statistically significant and therefore excluded from the final models. *Schistosoma haematobium* infection was included as a response variable (binary), with age (years), sex, microhaematuria, macrohaematuria, and all growth and nutritional indices, included as explanatory variables. Each morbidity indicator (i.e. microhaematuria, macrohaematuria, and all growth and nutritional indices) was also included in separate models, with age (years), sex and *S. haematobium* infection status as explanatory variables.

The risk of associated morbidity in relation to *S. haematobium* infection was estimated using prevalence ratios (PR). The PR was calculated as a ratio of the proportion of infected individuals with the associated morbidity (e.g. microhaematuria) to the proportion of uninfected individuals with the same morbidity. A morbidity indicator with a PR >1, suggested an increased risk of the associated morbidity from schistosome infection (Booth 1998). The method of attributable fraction (AF) was used to estimate the proportion of morbidity that could be attributed to *S. haematobium* infection. The population attributable fraction (AFp) and attributable fraction in the exposed (AFe) were used to estimate the morbidity attributable fractions in the whole study population and among schistosome-infected children respectively, according to Miettinen's formula (Miettinen 1974):

The AF in the infected population (exposed) was calculated as:

$$AFe = \frac{(RR - 1)}{RR} \tag{3.1}$$

The AF in the total population was calculated as:

$$AFp = Pe \times AFe \tag{3.2}$$

Here, RR is the risk ratio of morbidity associated with infection, and Pe is the prevalence of morbidity among the infected population.

As suggested by Booth (1998), RR can be substituted with PR in helminth epidemiological studies conducted at the cross-sectional level, as is the case with the current study (i.e. the baseline survey). AFs were only estimated for morbidity indicators with PR >1, showing a positive association with *S. haematobium* infection.

Treatment efficacy against *S. haematobium* infection was assessed by means of egg reduction rate (ERR) and cure rate (CR), limited to the cohort of children who were present for post-treatment efficacy check at follow-up surveys. These were defined as:

$$CR = \frac{number\ of\ children\ not\ excreting\ eggs\ at\ efficacy\ check}{number\ of\ children\ excreting\ eggs\ at\ baseline} \times 100\% \qquad (3.3)$$

$$ERR = \frac{arithmetic\ mean\ egg\ count\ of\ group\ (baseline - efficcay\ check)}{arithmetric\ mean\ egg\ count\ of\ group\ at\ baseline} \times 100\% \qquad (3.4)$$

For all analyses, approximate 95% confidence intervals (CI) were calculated using the modified Wald method (Agresti and Coull 1998), and p-values <0.05 were considered significant.

3.4 Results

3.4.1 Participant characteristics and survey dynamics

Of the 1502 children recruited, 794 (52.9%) were male. Median age was 3.5 years (IQR= 2.5–4.3; range= 0.5–5). Within this cohort, the youngest participant in whom *S. haematobium* infection was detected was a year old. Follow-up and infection rates at each survey time point throughout the study are shown in **Figure 3.2**.

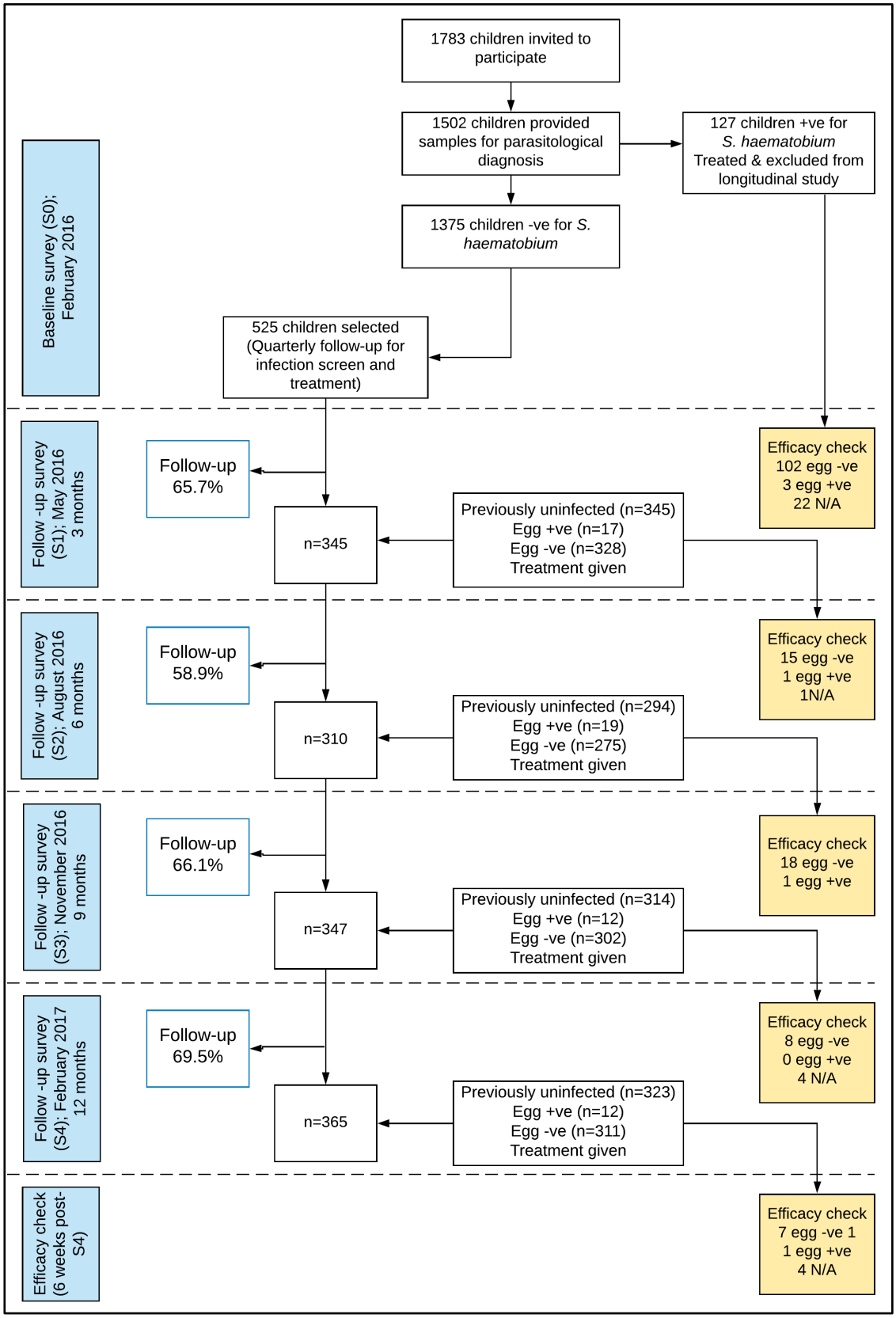

Figure 3.2: Flow chart showing participant follow-up and infection rates
+ve, positive; -ve, negative.

3.4.2 Baseline *S. haematobium* infection and age-dependent profiles

At baseline, 127 participants (8.5%; 95% CI= 7.2–10.0) were positive for *S. haematobium* infection based on egg counts. Children positive for infection were older when compared to those negative for infection (median age 4.0 versus 3.5 years; p= 0.001). There was no significant difference in infection prevalence between males and females; 8.9% (95% CI= 7.1–11.1) and 8.1% (95% CI= 6.3–10.4; p= 0.067) respectively. The overall mean infection intensity was 1.5 eggs/10 ml urine (95% CI= 0.8–2.2) and the majority of children, 119 (93.7%; 95% CI= 87.9–97.0) presented with light infections (<50 eggs/10 ml of urine) based on the WHO classification (WHO Expert Committee 2002). As shown in **Figure 3.3**, *S. haematobium* infection prevalence and intensity increased with age (p<0.001), supported by the age-dependent infection prevalence and intensity model.

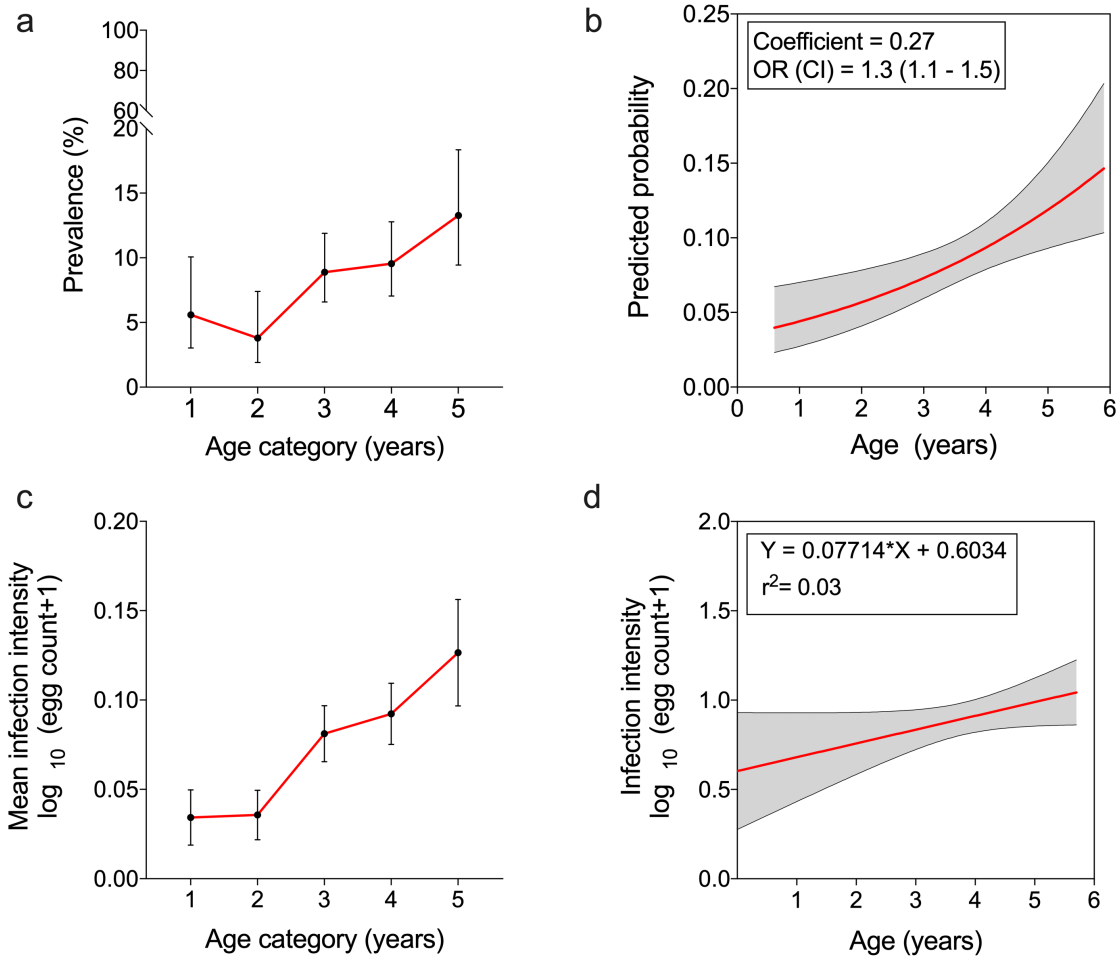

Figure 3.3: Distribution of *S. haematobium* infection prevalence and intensity with age
a) *S. haematobium* infection prevalence was associated with age (p <0.001), and b) the age-predicted probability of infection showed that infection prevalence increased with age (p= 0.002). c) *S. haematobium* infection intensity was associated with age (p <0.001), and d) the age-predicted infection intensity model showed that infection intensity increased with age. Error bars indicate 95% confidence intervals (panel a) or standard error of the mean (SEM) (panel c), and shaded areas indicate 95% confidence intervals (panels b and d).

3.4.3 Schistosome-related morbidity at baseline

Prevalence of urinary morbidity was 0.7% (95% CI= 0.3–1.5) for macrohaematuria, and 8.6% (95% CI= 6.9–10.6) for microhaematuria. Prevalence of malnutrition as measured by different indices were as follows: MUAC, 2.2% (95% CI= 1.4–3.2), mid-upper arm circumference Z-scores (MUACZ), 7.4% (95% CI= 6.0–9.1), and weight–for–height Z-scores (WHZ), 8.2% (95% CI= 6.8–9.9). Prevalence of underweight measured by weight–for–age Z-scores (WAZ) was 10.1% (95% CI= 8.5–11.9), and that by body mass index–for–age Z-scores (BAZ) was

8.8% (95% CI= 7.4–10.6). Prevalence of stunting by height–for–age Z-scores (HAZ) was 18.0% (95% CI= 16.0–20.3). Comparing infected versus uninfected children, prevalence of microhaematuria (43.5%; 95% CI= 34.8–52.6 versus 3.4%; 95% CI= 2.4–5.0 respectively; p <0.001) and stunting (27.0%; 95% CI= 19.9–35.6 versus 17.0%; 95% CI= 14.9–19.4 respectively; p= 0.009) was significantly higher among children with *S. haematobium* infection (**Figure 3.4**). Prevalence of malnutrition and being underweight by all the different indices (MUAC, MUACZ, WHZ, WAZ, and BAZ) were not significant when compared between schistosome positive and negative children (p >0.05) (see **Supplementary Figure 2 in Appendix A**).

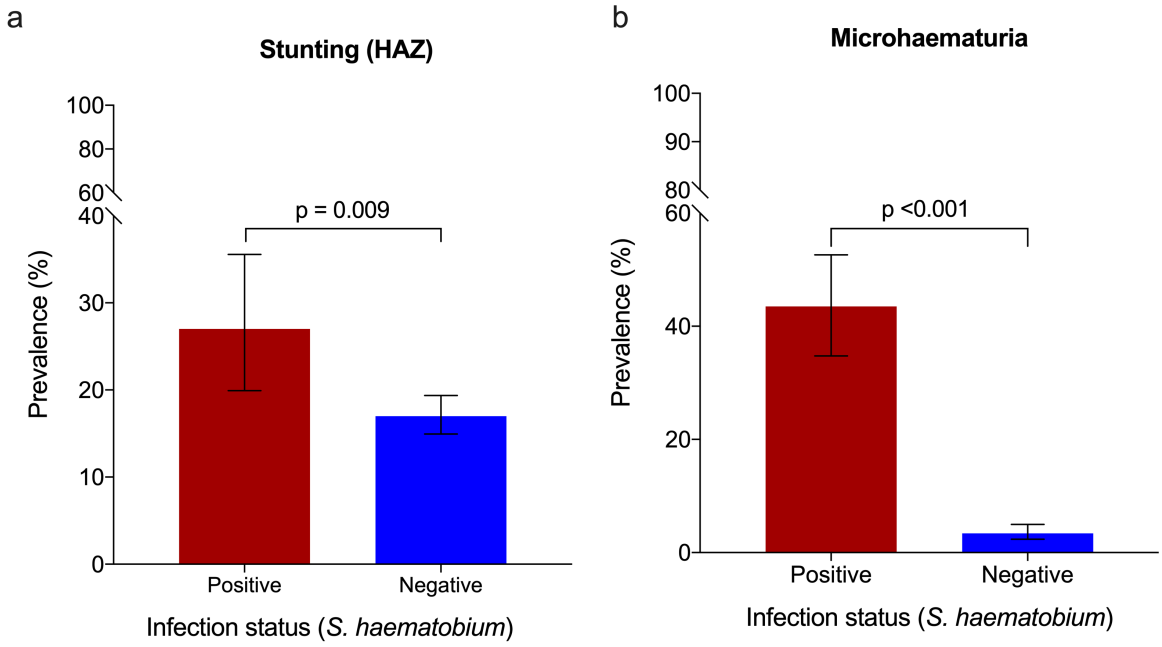

Figure 3.4: Prevalence of schistosome-related morbidity stratified by infection status
a) Stunting and b) microhaematuria were significantly higher amongst *S. haematobium* positive children. Error bars indicate 95% confidence intervals of the observed prevalence. p-values show test for the difference in prevalence of morbidity between the two groups (Fisher's exact test).

3.4.4 Morbidity attributable to *S. haematobium* infection

The risk of morbidity from *S. haematobium* infection was estimated using prevalence ratios (PR), based on the different morbidity indices considered. All morbidity markers but one, underweight by BAZ, had PR >1, indicating a significant association with *S. haematobium* infection (**Table 3.1**). However, since morbidities from schistosome infection are not specific and may relate to different physiological, biochemical and immunological processes, I determined how much of the detected morbidity was attributable to schistosome infection, limited to the indices with PR >1. As shown in **Figure 3.5**, results indicated that microhaematuria was the most dominant morbidity attributable to schistosome infection, both in infected children (92.0%) and at the population level (40.0%). Macrohaematuria on the other hand was highly attributable to schistosome infection in infected children (71.0%), but this was not the case in the total population (1.2%). Of the anthropometric markers, stunting was the most attributable to schistosome infection both in infected children (38.0%) and at the population level (10.3%).

Table 3.1: Prevalence ratios (PR) for schistosome-related morbidity

Morbidity	Diagnostic tool/index	PR (95% CI)
Microhaematuria	Urine dipsticks	12.6 (11.6-14.1)
Macrohaematuria	Visual inspection (colorimetry)	3.4 (1.9-5.4)
Stunting	HAZ	1.6 (1.05-2.31)
Malnutrition	WHZ	1.1 (0.9-1.4)
	MUACZ	1.5 (1.3-1.9)
	MUAC	1.3 (0.8-1.9)
Underweight	WAZ	1.4 (1.2-1.6)
	BAZ	1.0 (0.8-1.3)

PR, prevalence ratio; HAZ, height–for–age Z-scores; WAZ, weight–for–age Z-scores; BAZ, BMI–for–age Z-scores; MUAC, mid-upper arm circumference Z-scores; WHZ, weight–for–height Z-scores. 95% CI, 95% confidence interval.

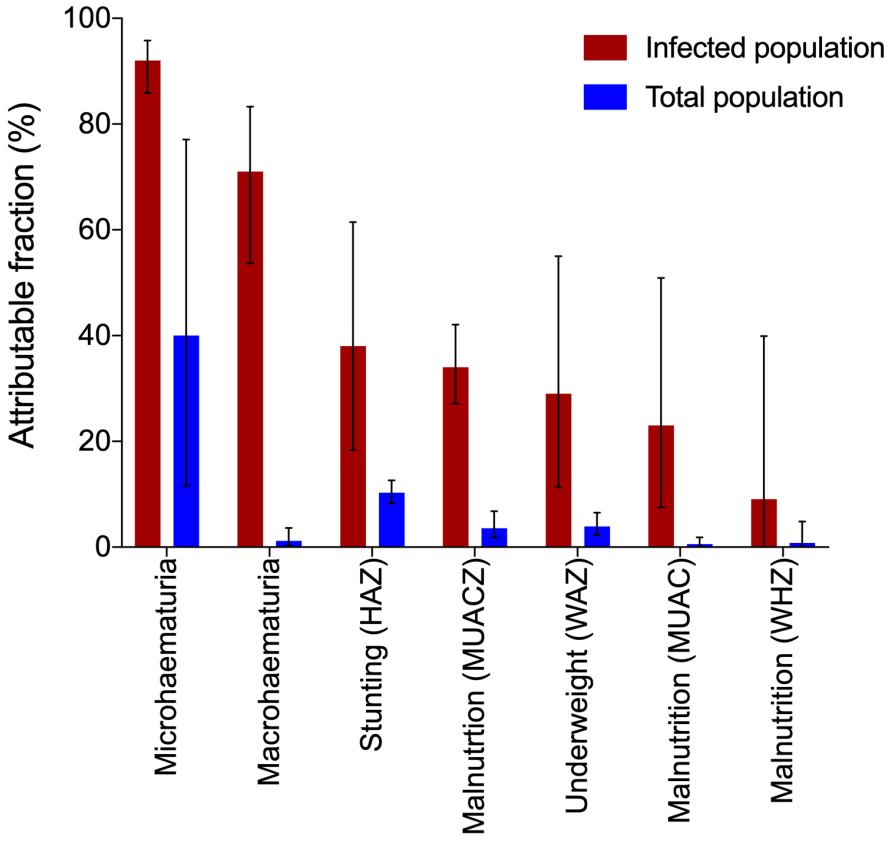

Figure 3.5: Estimated proportion of morbidity attributable to *S. haematobium* infection
HAZ, height-for-age Z-scores; MUAC, mid-upper arm circumference Z-scores; WAZ, weight-for-age Z-scores; MUAC, mid-upper arm circumference; WHZ, weight-for-height Z-scores. Error bars indicate 95% confidence intervals.

3.4.5 Risk of schistosome infection and morbidity

As shown in **Figure 3.6a**, multiple logistic regression analysis showed that with every unit increase in age, children were more likely to acquire *S. haematobium* infection (AOR= 1.4; 95% CI= 1.1–1.8; p= 0.005). Similarly, children who presented with microhaematuria were more likely to be positive for *S. haematobium* infection (AOR= 21.8; 95% CI= 11.7–40.7; p <0.001), compared to children who were negative for microhaematuria. Regarding schistosome-related morbidity, children with *S. haematobium* infection were 25 times more likely to present with microhaematuria (AOR= 25.6; 95% CI= 14.5–45.1; p <0.001), and two times more likely to be stunted (AOR= 1.7; 95% CI= 1.1–2.7; p= 0.014), compared to uninfected children (**Figure 3.6b**).

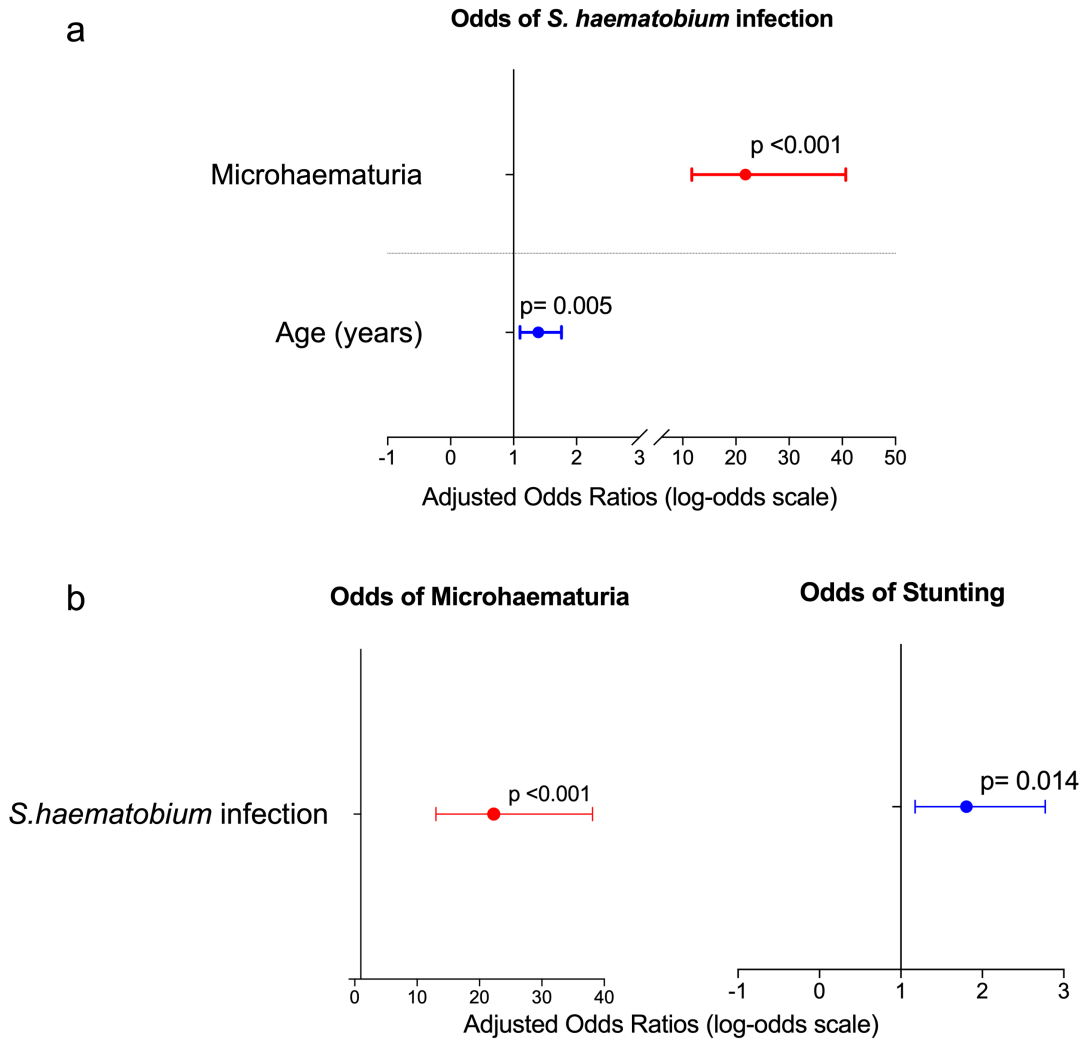

Figure 3.6: Odds of presenting with *S. haematobium* infection and morbidity
The forest plots show a) the odds of presenting with *S. haematobium* infection, and b) the odds of presenting with microhaematuria and stunting. Error bars indicate the 95% confidence intervals. Non-significant variables were excluded from the final multiple logistic regression model.

3.4.6 Incidence of infection and morbidity

The longitudinal cohort of 525 schistosome-negative children were followed quarterly for a year, and for each quarter, to determine their first schistosome infection and morbidity acquired in the previous 3 months. Annual incidence of *S. haematobium* infection was 17.4% (95% CI= 13.7–21.8) and the incidence of microhaematuria was 20.4% (95% CI= 15.8–26.0). *Schistosoma haematobium* incidence rates showed seasonal patterns. Incidence in the dry season was 4.9% in May (95% CI= 3.1–7.8) and 6.5% in August (95% CI= 4.1–9.9), while in

the rainy season incidence was 3.8% in November (95% CI= 2.1–6.6) and 3.7% in February (95% CI= 2.1–6.5). However, the difference in incidence rates between the dry (10.4%; 95% CI= 7.6–14.1) and rainy seasons (7.4% total; 95% CI= 5.0–10.9) was not significant (p= 0.175). The quarterly incidence of microhaematuria recorded was 2.0% in May (95% CI= 0.4–5.9), 2.8% in August (95% CI= 1.0–6.6), 13.3% in November (95% CI= 9.6–18.3), and 4.3% in February (95% CI= 2.2–8.1).

3.4.7 Treatment efficacy and effects of praziquantel treatment on morbidity

Children who received praziquantel treatment for schistosome infection were followed up at subsequent surveys (12 weeks post-treatment for all surveys and 6 weeks post-treatment for the last survey) for a post-treatment efficacy check. Treatment efficacy was calculated for all children found to be positive for infection at baseline and throughout the year for the longitudinal study. Of a total 187 children treated for infection (i.e. 127 at baseline and 60 from the longitudinal cohort), post-treatment data were available for 156 (follow up rate: 83.4%) [see **Figure 3.2**]. PZQ was efficacious in reducing *S. haematobium* infection, as indicated by the high CR (96.2%; 95% CI= 91.7–98.4, 150 egg-negatives) and ERR (97.3%; 95% CI= 85.0–100, mean egg intensity of 21.60 eggs/10ml at pre-treatment versus 0.58 eggs/10ml at post-treatment). None of the children included in the current study reported adverse side-effects from treatment, and this was confirmed by field clinicians as described in **Chapter 2**.

The effects of treatment on morbidity was determined for participants from the baseline survey and the longitudinal cohort. Data for microhaematuria pre- and post-treatment was available for 78 of the 127 *S. haematobium*-positive cases identified at baseline. Within this cohort, 42.3% (95% CI= 32.0–53.4) were positive for microhaematuria and this declined significantly post-treatment (10.3%; 95% CI= 5.1–19.2; p <0.001).

A pooled analysis of egg-positive children identified in the longitudinal cohort was done to determine the dynamics of microhaematuria before, during and after infection. Of the total 60 new infections detected throughout the follow up period, microhaematuria was detected at least once, among 18 individuals; 6 (33.3%) pre-infection, 11 (61.1%) during infection, and 2 (11.1%) post-treatment. In a majority of individuals (61.1%), microhaematuria coincided with the detection of *S. haematobium* infection (within 3 months), and had resolved by the next survey at 3 months post-treatment of infection (**Figure 3.7**).

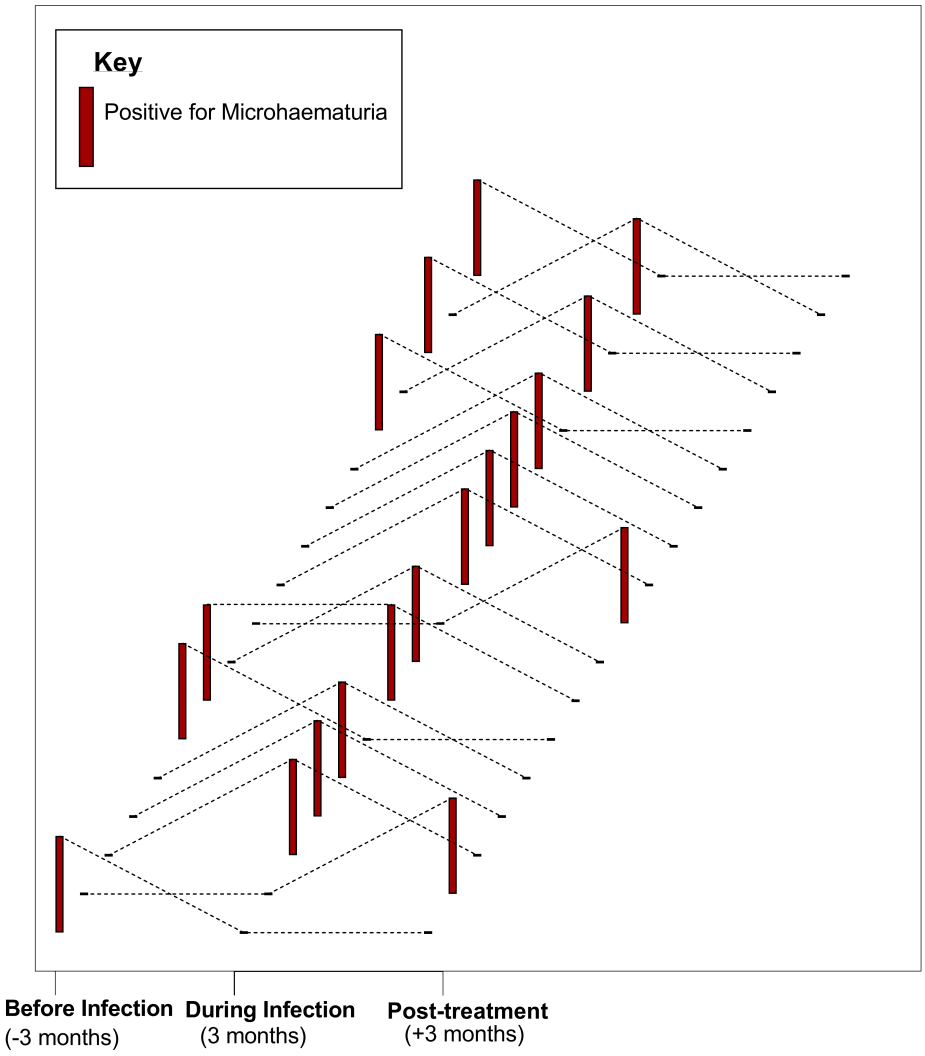

Figure 3.7: Impact of *S. haematobium* infection and treatment on morbidity (microhaematuria)
Microhaematuria status for 18 individual participants is shown at three time points: before, during and post-infection (post-treatment). Each data set (dotted line path) represents one individual. Tall, red bars indicate positive microhaematuria, and a black dash indicates negative microhaematuria at specific time points.

3.5 Discussion

Contrary to the previously held assumption of a low risk to schistosome infection in PSAC (Sacko *et al.,* 2011), the evidence of schistosome infection in infants and young children is increasingly being recognised (Freer *et al.,* 2018). The operational challenges with diagnosis and the lack of adequate knowledge on risks, burden estimates, and health impacts of infection and treatment in PSAC are a major challenge for control efforts. In this chapter, I determined in a cohort of PSAC, the incidence and dynamics of the first urogenital schistosome infection and morbidity, as well as the associated risks and health impacts of the infection. The findings showed that PSAC present with schistosome infection (estimated by egg count) and morbidity (determined by microhaematuria, growth and nutritional indices) from an early age. This infection and associated morbidity can be detected early in PSAC using parasitology and dipstick techniques (microhaematuria) within 3 months of the first infection, and is resolved after treatment. Chronic growth/nutrition-related morbidity can help identify high-risk groups and measure the health impacts of infection and treatment in endemic areas.

The observed prevalence of *S. haematobium* infection (8.5%) is comparable to *S. haematobium* prevalence rates reported in other studies conducted in PSAC. Prevalence rates of 11.2% have been reported in Ghana (Bosompem *et al.,* 2004), 10.7 % in Malawi (Poole *et al.,* 2014), and in Zimbabwe, 13.5% (Wami *et al.,* 2015), 6.7% (Wami *et al.,* 2016) and 13.3% (Mutsaka-Makuvaza *et al.,* 2018, Mutsaka-Makuvaza *et al.,* 2019). Consistent paediatric schistosomiasis research work carried out by our research group (Parasite Immuno-epidemiology Group) in Zimbabwe, where PSAC are screened and all infected children treated with PZQ, may contribute to the lower prevalence rate observed in the current study. Through our studies, *Schistosoma haematobium* prevalence rates in PSAC have progressively declined over the years from 29% in the year 2000, to 13.5% as of 2015 (Woolhouse *et al.,* 2000, Mutapi *et al.,* 2011, Wami *et al.,* 2015). In addition, the role of increased awareness/education for caregivers and parents of PSAC through such studies cannot be overemphasised. Education of

the caregiver (through interactive materials) is known to change customs and behaviours that put PSAC at risk of schistosome infection (WHO Expert Committee 2002, Beanland *et al.,* 2006). This may have contributed over time to reduced risk/exposure to schistosome infection, hence a lower infection prevalence. That notwithstanding, PSAC present with predominantly light infections (Stothard *et al.,* 2011, Colley *et al.,* 2014, Wami *et al.,* 2014) and parasitological egg counts can underestimate the prevalence of schistosome infection (Wami *et al.,* 2014, Le and Hsieh 2017). Thus, it is likely that the prevalence found in the current study may be higher if the more sensitive serological diagnostic tools are used (Wami *et al.,* 2014).

In agreement with previous findings (Woolhouse *et al.,* 2000, Kanamura *et al.,* 2002, Lengeler *et al.,* 2002, van Dam *et al.,* 2004, Wami *et al.,* 2014), infection prevalence and intensity increased as children grew older. The prevalence of infection and its age-associated trend in PSAC is an indication that infection, if left untreated, will likely lead to severe morbidity later in life (Stothard *et al.,* 2011).

The interpretation of biomarkers of schistosome-related morbidity in the light of coinfections and comorbidities in endemic areas is complex, and calls for better-defined schistosome morbidity indicators in PSAC. The majority of such morbidity indicators are non-specific and relate to various physiological, biochemical and immunological processes (Webster *et al.,* 2009). In the current study, the prevalence of morbidity and how much of this was attributable to *S. haematobium* infection was determined. Microhaematuria was the most dominant marker of schistosome-related morbidity, and children with *S. haematobium* infection were more likely to present with microhaematuria and vice versa. This agrees with previous findings from Zimbabwe (Wami *et al.,* 2015) and Nigeria (Salawu and Odaibo 2014), on the significance of microhaematuria as a point-of-care field marker of morbidity for urogenital schistosomiasis in PSAC. Haematuria results from epithelial damage when parasite eggs of *S. haematobium* are passed through to the bladder lumen (Wilkins *et al.,* 1979, Gryseels *et al.,* 2006, Colley *et al.,*

2014) and correlates with infection intensity (Ismail *et al.,* 2014, Ochodo *et al.,* 2015). Based on this, the field utility of microhaematuria in urogenital schistosomiasis has been validated in schistosome-endemic areas (Anosike *et al.,* 2001, King and Bertsch 2013) and its applicability and sensitivity decreases with age (Akogun and Obidiah 1996, Etard 2004b). Therefore, as observed in the current study, microhaematuria is likely to perform better as a diagnostic tool for *S. haematobium* infections in PSAC, than in SAC and adults.

The prevalence of stunting and malnutrition, and their association with *S. haematobium* infection in this cohort was also investigated. The results showed that of the growth and nutritional indices, stunting, as measured by HAZ, was the most dominant marker attributable to schistosome infection. Moreover, children with *S. haematobium* infection were more likely to have stunted growth, compared to uninfected children. To the best of my knowledge and at the time of writing this thesis, this is the first study showing the relationship between *S. haematobium* infection and chronic growth failure, i.e. stunting, in PSAC. Studies on polyparasitism (Mupfasoni *et al.,* 2009, Bustinduy *et al.,* 2013) and limited schistosome studies on *S. mansoni* (Assis *et al.,* 1998) and *S. japonicum* (Coutinho *et al.,* 2006) have however documented this effect in older children. In agreement with the findings of the current study, stunting as detected in older children and adolescents is believed to be the result of chronic parasite-induced inflammation which persists during childhood (Friedman *et al.,* 2005). However, causality is difficult to establish in the case of my current findings, due to the lag time between the initial infection and the time at which growth failure was measured, as well as the impact of confounding factors such as diet and coinfections on overall health. There is the need for longer-term studies investigating the impact of treatment on growth and development measures in PSAC. Statistical modelling suggests that with early, repetitive treatment of infection before the school age (i.e. 6 years old), “catch-up growth” can be effectively facilitated (Gurarie *et al.,* 2011).

While the prevalence and intensity of schistosome infections have been described in PSAC from several African countries, studies describing the incidence of schistosome infection in PSAC are lacking. Moreover, although the first infection typically occurs at the preschool age in schistosome-endemic areas, knowledge on the earliest events that occur during the first infection is lacking; in particular is when infection and morbidity can occur and be detected following exposure in this age group. In the current study, I document the incidence of the first *S. haematobium* infection and morbidity in this PSAC population. This group of children had not been previously infected or treated for schistosomiasis (assessed by health questionnaire and clinical records), and were confirmed schistosome-negative (by egg count) at baseline, prior to recruitment into the longitudinal study. Results showed evidence of the first *S. haematobium* infections and morbidity (as measured by microhaematuria), every 3 months. This is an indication of the applicability of currently available tools, i.e. parasitology egg counts and urine dipstick techniques), to screen for early infection and morbidity in PSAC living in schistosome-endemic areas. The incidence of microhaematuria and the associated attributable fraction observed, demonstrates that even for the very first schistosome infection in PSAC, clinical symptoms and substantial morbidity develops early in infection (Sacko *et al.,* 2011, King 2015). In the case of the current study, this was reflected as microhaematuria, which is an indication of active bladder/ureteral lesions and blood losses (Wilkins *et al.,* 1979, Gryseels *et al.,* 2006, Colley *et al.,* 2014) even in light intensity schistosome infections, as is the case in PSAC (Stothard *et al.,* 2011, Colley *et al.,* 2014).

The seasonal pattern of infection incidence observed is in agreement with the fact that higher temperatures and seasonal rainfall patterns affect transmission, by favouring survival of intermediate snail host populations as well as parasite development stages in the snail (Chingwena *et al.,* 2002, McCreesh and Booth 2014, Pedersen *et al.,* 2014). During the dry seasons, snail vectors and larval schistosomes become concentrated at permanent and slow-moving water sources, increasing the risk of infection in both the snail and human hosts

(Woolhouse and Chandiwana 1989, Rollinson *et al.,* 2001, Monde *et al.,* 2016). In addition, snail generation times decrease, and the time from larval infection to production of infective cercariae by snails also decreases with increasing temperatures (McCreesh and Booth 2014). Observations from the fieldwork conducted for the current study also indicates that during the wet seasons, households are less reliant on infective water sources for chores, and children are less likely to visit such water bodies for recreational purposes. The incidence dynamics observed in the current study confirm that conditions for schistosome exposure in PSAC vary, and operational plans for the control of infection in this age group should take such dynamics into consideration.

In terms of the impact of treatment on infection and morbidity, the observed ERR and CR showed that a single standard dose of PZQ was effective against *S. haematobium* infection in this PSAC population. This is consistent with reports on PZQ efficacy for schistosomiasis in PSAC (Mutapi *et al.,* 2011, Coulibaly *et al.,* 2017, Coulibaly *et al.,* 2018). In addition, studies have shown that PSAC tolerate PZQ well, with reports of very few side-effects, including abdominal pain, vomiting, fatigue, and diarrhoea that are self-limiting and resolve within 24 hours (Sousa-Figueiredo *et al.,* 2010, Mutapi *et al.,* 2011, Namwanje *et al.,* 2011, Coulibaly *et al.,* 2017, Coulibaly *et al.,* 2018). Thus, it is in line that in this cohort no adverse side-effects were reported. Microhaematuria occurred rapidly within 3 months of exposure to infection and resolved within 3 months after treatment with PZQ. This finding is in line with the fact that microhaematuria is associated with *S. haematobium* infection (Wilkins *et al.,* 1979, Gryseels *et al.,* 2006, Colley *et al.,* 2014), and that treatment with PZQ reduces schistosome-related microhaematuria, as used in post-treatment monitoring during large-scale chemotherapy for SAC (Koukounari *et al.,* 2007, Webster *et al.,* 2009, King and Bertsch 2013). The observed impact of treatment is an important finding for the undelayed/early screening and treatment of schistosome infections in PSAC, to avert accumulative morbidity which can affect overall health.

Previous epidemiological schistosome studies conducted in PSAC have been limited to estimating the prevalence and intensity of infection and morbidity. In addition to adding to these estimates, the major strength of the current study is that it follows a natural time-course to detect the first *S. haematobium* infections in a preschool cohort, that had not been previously infected or treated for schistosome infection. It determines how quickly the very first infection can be detected following exposure, when infection-related morbidity develops and can be detected, as well as the impacts of treatment on infection and morbidity. Although the impact of schistosome infection on the health of children is likely to be greater than those explored here, interpretation of the above findings must be done considering the limitations of some of the study methods. A limitation of the parasitological detection of infection is that, some light infections may have been missed, resulting in misclassification and underestimation of the infection rates. Nonetheless, the current study allows comparison with other studies and contributes to knowledge on infection and morbidity burden and dynamics, while parasitological methods remain the predominant schistosome diagnostic in PSAC.

3.6 Conclusions

The current study demonstrates for the first time, incidence of the first schistosome infection and morbidity in PSAC, as estimated by egg counts and microhaematuria respectively. This infection and morbidity occur rapidly and can be detected within 3 months of first infection. More importantly for child health and development, treatment of schistosome infection leads to a significant resolution in microhaematuria, and this occurs within 3 months of PZQ treatment. In schistosome-endemic areas, chronic growth and nutrition-related morbidity such as stunting and malnutrition, are attributable to *S. haematobium* infection in PSAC. Taken together, the findings of this chapter add to the infection and disease burden estimates in PSAC, while describing the events that occur early during the first schistosome infection in endemic areas. The findings further demonstrate the ability of existing diagnostic and morbidity tools, to quantify and monitor early infection and morbidity in PSAC, especially

when used together. Undelayed screening and treatment of schistosome infections in PSAC is essential to avert accumulative morbidity, which can affect overall health. Operational and economic plans for control must take into account incidence rates and dynamics of infection and morbidity in this age group, as highlighted here.

Chapter 4 Impact of praziquantel treatment on *Schistosoma haematobium* infection and reinfection rates in preschool-aged children with no history of infection

4.1 Introduction

Praziquantel (PZQ) is the antihelminthic drug of choice for treating schistosomiasis (World Health Organization 2020b), and is safe and efficacious in preschool-aged children (PSAC), i.e. children who are 5 years old and below (Mutapi *et al.,* 2011, Coulibaly *et al.,* 2017, Montresor and Garba 2017, Coulibaly *et al.,* 2018). There is increasing evidence that PZQ treatment of schistosome infections may have additional benefits beyond the removal of infection. PZQ treatment in older children and adults can induce immune responses associated with protection against reinfection (Black *et al.,* 2010a, Black *et al.,* 2010b, Bourke *et al.,* 2013). Recently, our research group (Parasite Immuno-epidemiology Group) demonstrated this phenomenon in Zimbabwean PSAC, showing an increase in post-treatment anti-parasite immunoglobulin E titres associated with resistance to reinfection (Rujeni *et al.,* 2013). The mechanistic pathways of this long-term effect include the enhancement of schistosome-specific immune responses, by removing the immuno-suppressive effects of infection (Mitchell *et al.,* 2011, Nausch *et al.,* 2015, Schmiedel *et al.,* 2015), and introducing parasite-specific antigens to the immune system to induce and accelerate protective immune responses (Mutapi *et al.,* 2005, Mutapi *et al.,* 2008, Bourke *et al.,* 2013). One of the changes underlying the progression of schistosome-exposed individuals from a phenotype susceptible to infection/reinfection to a phenotype resistant to reinfection, is in the ratio of effector versus regulatory immune responses (Nausch *et al.,* 2011). This mechanistic change has been shown to occur naturally with infection over longer periods, as well as following curative treatment of schistosome infections, but more rapidly with treatment (Mutapi *et al.,* 2005, Watanabe *et al.,* 2007, Nausch *et al.,* 2011).

Schistosome infections start to become chronic after about 12 weeks post-infection, and with stronger immuno-suppressive effects in the host (Pearce and MacDonald 2002, Colley and Secor 2014). Therefore, given the current practise of mass drug administration programmes (MDA) where the most frequent treatment will be on an annual basis (World Health Organization 2006), inclusion of PSAC in MDA programmes will imply treating a majority of children who have had to carry infections for a longer time, possibly allowing the infection to become chronic and with debilitating effects (Secor 2015). However, pending roll out of the paediatric PZQ (undergoing phase III clinical trials) for inclusion in MDA, the currently recommended approach for this age group is treatment upon diagnosis, and on a case-by-case basis (World Health Organization 2011, Bustinduy *et al.,* 2016a). Therefore, knowledge of optimal screening and treatment times to reduce infection, and to maximise the health benefits of treatment in terms of reinfection to avert severe or long-term morbidity, will be important for the control of paediatric schistosomiasis.

In the preceding chapter (**Chapter 3**), I demonstrated the incidence of the first schistosome infection and morbidity in PSAC, and the impact of treatment on the reversal of early morbidity. This reaffirms the need for early detection and treatment in this age group, to prevent the development of severe morbidity, even with the very first infection. My findings and those from previous epidemiological field studies in PSAC (Woolhouse *et al.,* 2000, Bosompem *et al.,* 2004, Odogwu *et al.,* 2006, Mutapi *et al.,* 2011, Poole *et al.,* 2014, Wami *et al.,* 2015, Kemal *et al.,* 2019, Mutsaka-Makuvaza *et al.,* 2019, Sacolo-Gwebu *et al.,* 2019), further heighten the need for optimum screening and treatment strategies to improve the health benefits associated with PZQ treatment, in terms of reinfection and reducing long-term morbidity. Here, I postulated that a single round of PZQ treatment, early in the course of the first schistosome infection, may help overcome the effect of regulatory responses, and thus allow the development of resistance against reinfection. This is based on the current understanding of the development of schistosome-protective immune responses with

treatment, and the fact that the rate of acquired resistance to schistosome reinfections is dependent on exposure history and repetitive rounds of cure, as seen with treatment of chronic infections (Black *et al.,* 2010a, Black *et al.,* 2010b).

In the current study, I aimed to determine the operational health benefits of regular quarterly screening and treatment of schistosome infections (upon first infection), in terms of reducing subsequent new infections and reinfections in PSAC. This aim was two-fold; first was to determine in a cohort of PSAC experiencing their first schistosome infection, the impact of regular quarterly screening and treatment on subsequent infection and reinfection rates. Secondly, the reinfection rates and intensity observed following a single PZQ treatment of the first schistosome infection in PSAC, was compared to that observed with the treatment of chronic infections. Knowledge of a routine screen-and-treat strategy in PSAC, will optimise the early detection and treatment of schistosome infections before they become chronic, and maximise the health benefits of PZQ treatment, in terms of reduced reinfection and morbidity.

4.2 Hypotheses

(i) Regular quarterly screening and treatment of schistosome infections (upon first infection) in PSAC, results in reduced rates of subsequent new infections.

(ii) In previously uninfected PSAC, a single PZQ treatment of the first schistosome infection is associated with resistance to reinfection, similar to or greater than that observed following the treatment of chronic infections. This can be optimised to prevent severe schistosome-related morbidity in PSAC.

4.3 Methods

4.3.1 Study design, population and site

The current study followed a 2-year longitudinal infection–treatment–reinfection study design, including a subset of 633 PSAC (age range 1–5 years), who met the inclusion criteria for the larger paediatric urogenital schistosomiasis study described in **Chapter 2.** The subset of children included in the current study were recruited form the Chihuri, Madziwa and Mupfure villages.

The current study was designed to detect the impact of antihelminthic treatment with PZQ on infection and reinfection rates in two groups of schistosome-infected PSAC, i.e. a group of children experiencing their first schistosome infection, and a group of children with chronic infections. A cross-sectional study was conducted at baseline to screen children for schistosome infection, followed by a 2-year longitudinal study to determine infection and reinfection rates. The main aspects of the study were: (i) in the first year, to determine the impact of treating chronic *S. haematobium* infection on reinfection rates, and to determine and treat first schistosome infections among previously uninfected children, (ii) in the second year, to determine the impact of treating the first *S. haematobium* infection on reinfection rates, at least 12 months post-treatment. Further reinfection rates from previously treated chronic infections at baseline was also to be determined. To be included in the longitudinal cohort of children to be followed up quarterly in the first year, children had to meet an additional criterion of being diagnosed negative for *S. haematobium* infection by egg count at baseline. A schematic diagram of the study design is shown in **Figure 4.1**.

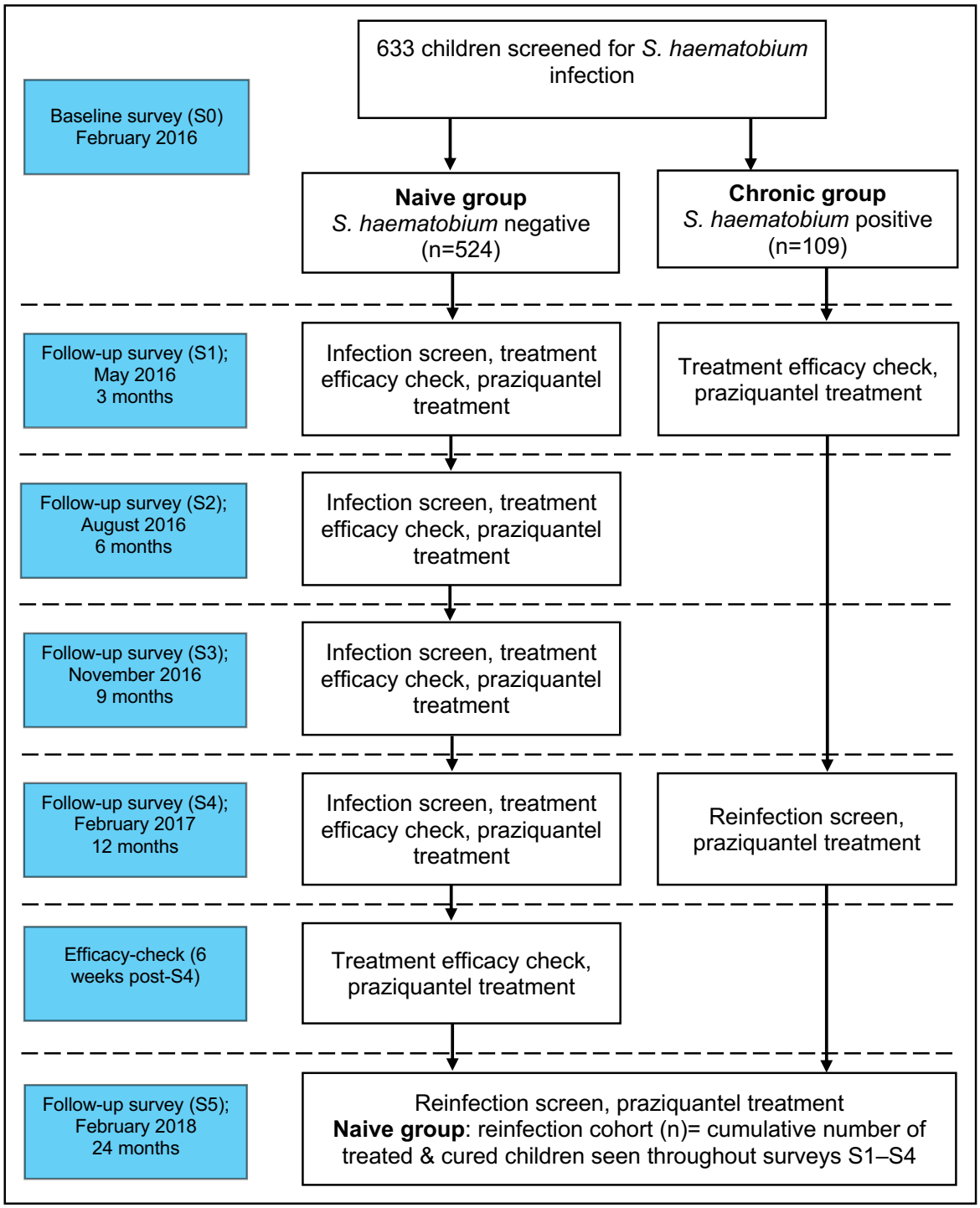

Figure 4.1: Schematic diagram of study design

At baseline (S0), 633 eligible children were screened for *S. haematobium* infection by parasitology (egg count). Metadata on socio-demography (including age and sex) gathered from questionnaires administered at the time of recruitment were used (described in **Chapter 2**). Following this, children confirmed positive for *S. haematobium* infection by egg count (n=109) were treated with a single dose of PZQ at the standard 40 mg/kg body weight (as described in **Chapter 2**), and formed the chronic group that was followed up annually for post-treatment reinfections. This group was considered "chronic" because they had no history of antihelminthic PZQ treatment prior to diagnosis at the baseline survey (assessed by health questionnaire and clinical records, as described in **Chapter 2**). Thus, children who were at least a year old (age range 1–5 years) in this group, were likely to have had infections for at least 12 weeks, when infection starts to become chronic (Pearce and MacDonald 2002, Colley and Secor 2014). Children confirmed negative for *S. haematobium* infection (n=524) formed the naive group that was followed up quarterly for their first *S. haematobium* infection and treatment in the first 12 months, and in whom reinfection rates would be detected a year later. This group was considered "naive" because they had no history of schistosome infection or antihelminthic PZQ treatment (assessed by health questionnaire and clinical records, as described in **Chapter 2**).

For 12 months, the naive group was followed up every quarter (surveys S1, S2, S3, and S4) for parasitology to detect their first *S. haematobium* infection. Both experimental and previous field studies show that the 3-month period is the maximum time for new *S. haematobium* infections to reach patency, and thus be detectable by egg excretion in urine (Webbe and James 1971, Smith *et al.,* 1976, Wami *et al.,* 2016). At each survey time point, all children who were positive for schistosome infection were treated with a single standard dose of PZQ (40 mg/kg). A post-treatment efficacy check by parasitology was carried out for all previously treated children at each subsequent follow-up survey (3 months after each previous survey for S1–S3, and at 6 weeks post-treatment at S4).

To determine the impact of PZQ treatment on reinfection, previously treated children in the chronic group, were followed up at 12 months and at 24 months (S4 and S5 respectively) for parasitology. Children previously treated for new schistosome infection in the naive group (from S1–S4) were followed up 12 months later (i.e. at 24 months at S5) for parasitology. In the naive group, egg-positive children identified at each survey who received treatment and were confirmed egg-negative at each subsequent follow-up survey, formed the cumulative reinfection cohort followed up at S5. At the end of the study, all children positive for schistosome infection were offered treatment.

4.3.2 Sample collection and processing

Urine and stool samples were collected for parasitological diagnosis of *S. haematobium* infection (all survey time points) and to exclude *S. mansoni* and soil transmitted helminths (baseline survey only) respectively. Details of sample collection and parasitological diagnosis are as previously described in **Chapter 2**.

4.3.3 Compliance assessment

Compliance as used in the current study, was defined as presence at each consecutive survey, and having provided the required parasitological samples for diagnosis or post-treatment efficacy check. If previously treated for *S. haematobium* infection, compliance also constituted being confirmed negative at post-treatment efficacy check, with no repeat PZQ treatment. None of the participants included in the current study received a repeat dose of PZQ treatment.

4.3.4 Data handling and statistical analyses

Data were analysed using SPSS version 22 (IBM Corp.), and graphs were prepared with GraphPad Prism version 7.02 (GraphPad Software, Inc.). Descriptive statistics were used to summarise measures of demography and infection. Continuous data are presented as mean ± standard deviation (SD), and categorical data are presented as absolute numbers and percentages. Infection intensity was expressed as the arithmetic mean egg count/10 ml of at

least two urine samples collected on three consecutive days. To meet the normality assumption of parametric statistics, infection intensity data was log-transformed ($\log_{10}$ [egg count + 1]). To determine the differences in (re)infection rates and infection intensity between two groups, Fisher's exact test and the t-test were used to test for differences in categorical and continuous variables respectively. For all analyses, approximate 95% confidence intervals (CI) were calculated using the modified Wald method (Agresti and Coull 1998), and p-values <0.05 were considered significant.

Infection and reinfection rates at a specified time, were calculated as the ratio of the number of infected participants to the number of participants seen at that time point, expressed as a percentage. Infection and reinfection rates were limited to compliant cohorts of both the naive and chronic groups. For the naive group, the rate of new infections at S4 was calculated as the cumulative number of infections over the 12-month quarterly follow-up period (S1-S4; 1year).

The force of infection (FoI) was calculated as the number of *S. haematobium* infections acquired at 3-month intervals, expressed as the number of new infections per unit time. FoI was calculated using the expression:

$$\lambda = \frac{[-\ln(1 - x(t))]}{t} \tag{4.1}$$

Where, λ is the FoI, t is the time (expressed in years), and x(t) is the incidence or proportion of infection at the time, t. This was to determine the rate at which susceptible children in the naive group acquired schistosome infection.

In order to determine the amount of time children were at risk of acquiring schistosome infection, the child life-years of infection was determined. Using the principle for estimation of person-years in a cohort (Vandenbroucke and Pearce 2012), the child life-years of infection was defined as the actual time at risk of *S. haematobium* infection in years, at specified time intervals or throughout the study period (cumulative). This was calculated as the number

infected at a particular time, multiplied by the risk-period (time to onset of infection) when the infection was acquired. The onset of infection was estimated as the midpoint of the time interval between being uninfected and becoming infected (i.e. 3 months) (Rothman *et al.,* 2008). Therefore, a risk-period of 1.5 months for each survey time point was used.

4.4 Results

4.4.1 Participant characteristics and survey dynamics

Overall, 633 children (aged 1–5 years old) were recruited for the current study, with a mean age of 3.7 years ± 1.0. Of these, 337 (53.2%) were male and 296 were female (46.8%). At baseline, 524 children negative for *S. haematobium* infection formed the naive group, and 109 children positive for *S. haematobium* infection formed the chronic infection group. The total number of children recruited, their demographics, and the number of children followed up at each survey throughout the first year are shown in **Table 4.1**.

Table 4.1: Participant characteristics and survey statistics for the first 12 months

Variable	Naive		Chronic
Age in years (mean ± SD)	3.7 ± 0.9		3.79 ± 1.1
Male	275 (52.5%)		62 (56.9%)
Female	249 (47.5%)		47 (43.1%)
Baseline participation (S0)	524 (100%)		109 (100%)
Participation in subsequent surveys *			
	Total	**Without infection**	
3 months (S1)	421 (80.3%)	421 (80.3%)	109 (100)
6 months (S2)	324 (77.0%)	308 (73.2%)	–
9 months (S3)	269 (83.0%)	236 (76.6%)	–
12 months (S4)	236 (87.7%)	196 (83.1%)	87 (79.8%)

The table summarises participant demographics and participation numbers in each study group at each survey timepoint. * The percentage of children followed up at each survey timepoint was calculated as the proportion of participants seen at each previous timepoint. Total= all children seen at a specified time point, including previously treated children (from previous time points) followed up for post-treatment efficacy check. Without infection= number of children seen, including only children who had never tested positive at previous timepoints throughout the follow-up period.

4.4.2 Schistosome infection dynamics in the first 12 months

In the first 12 months, incidence of first *S. haematobium* infections was recorded every quarter in the naive group, as indicated by the force of infection and the steady decline in the proportion of uninfected children (**Figure 4.2 a and b**). As shown in **Figure 4.2c**, the quarterly *S. haematobium* infection rates were 4.0% (95% CI: 2.5–6.4) at 3 months (S1), 5.8% (95% CI: 3.7–9.1) at 6 months (S2), 5.1% (95% CI: 2.8–8.8) at 9 months (S3), and 6.1% (95% CI: 3.4–10.5) at 12 months (S4).

At each survey time point, all *S. haematobium*-positive children were offered treatment with PZQ. Without quarterly screening and treatment of infections and assuming no natural loss in infections with time, the rate of new infections increased with time, showing a linear trend (**Figure 4.2c**). The impact of quarterly screening and early treatment of new infections on the number of child life-years of infection (the actual time at risk of *S. haematobium* infection in years) is shown in **Figure 4.2d**. Analysis showed a steady decline in the number of child life-years of infection with quarterly screening and treatment, in contrast to that observed assuming no treatment with time (i.e. increased with time). The number of child life-years of infection at the end of the 12 months would have been a cumulative 7.4 years without treatment, whilst that observed with quarterly screening and treatment was 1.5 years.

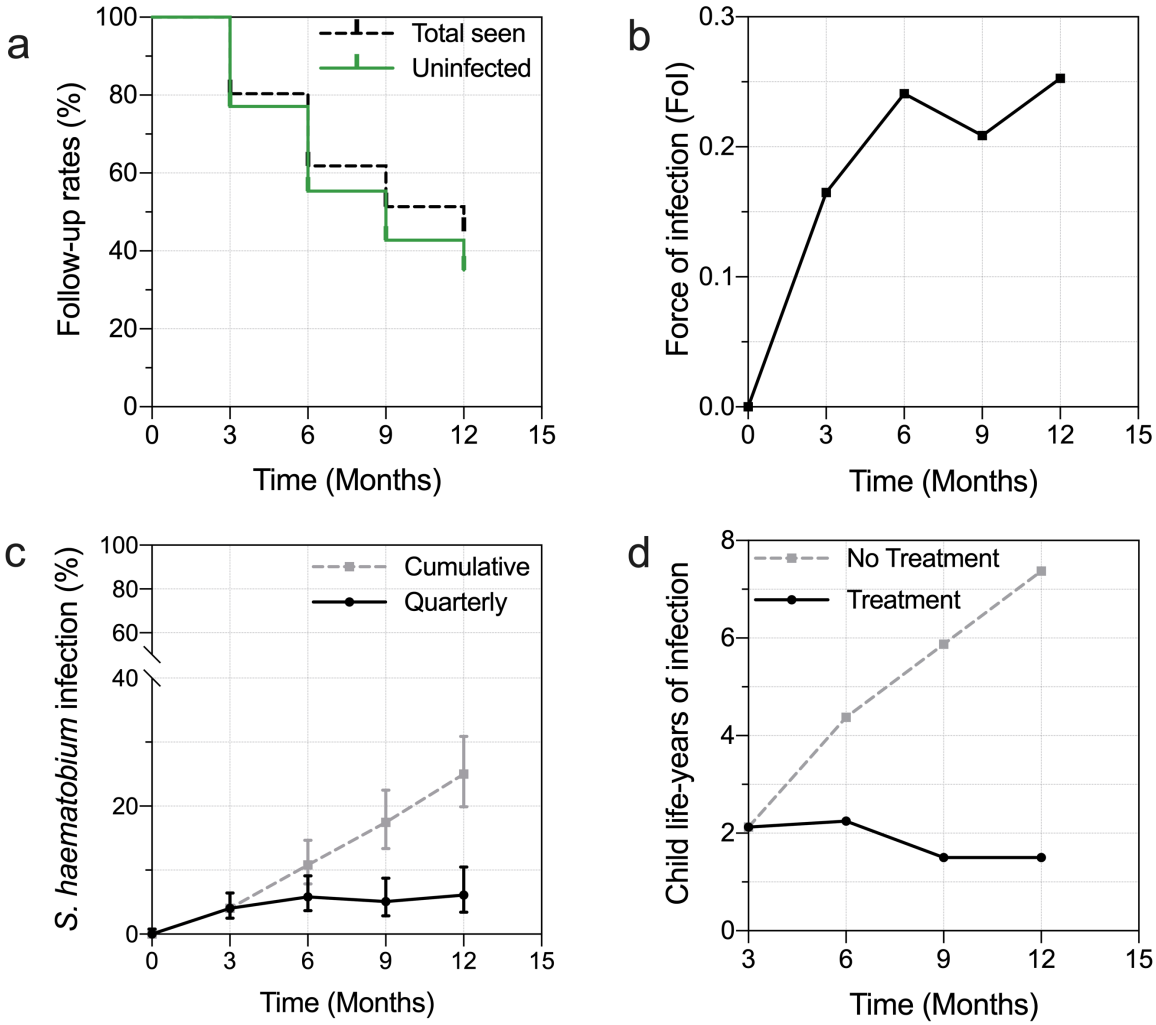

Figure 4.2: *S. haematobium* infection and treatment dynamics in the first 12 months; naive group
a) Kaplan–Meier plot showing the decline in the proportion of children followed up, and the decline in the proportion of uninfected children with time. b) Force of *S. haematobium* infection over 12 months. c) *S. haematobium* infection rates over time, comparing infection rates from quarterly screen and treatment of infections, with the cumulative prevalence rates assuming no natural loss in infections with time. d) Child life-years of *S. haematobium* infection over time, with and without quarterly treatment. Error bars indicate the 95% confidence intervals.

4.4.3 Schistosome infection and reinfection rates at 12 and 24 months

In the naive group, the annual rate of first infections was calculated as a proportion of the cumulative number of new infections to the total number of children followed up at 12 months, limited to the compliant group of children only. At 12 months, the cumulative number of egg-positive children treated and followed up at each consecutive survey was 52, with a total 236 children remaining/followed up (see **Table 4.1**)

To determine reinfection rates, previously treated compliant children from the chronic group at baseline, and previously treated compliant children from the naive group identified within the first 12 months, served as the reinfection cohorts for each group. The reinfection cohort from the chronic group was followed up at 12 months (S4) and at 24 months (S5), and the reinfection cohort from the naive group were followed up at 24 months (S5). In both groups, all previously treated children who were present for post-efficacy check were negative for *S. haematobium* infection, and children were excluded from the reinfection cohorts only as a result of losses to follow-up. **Figure 4.3** shows the annual infection and reinfection rates recorded in the naive and chronic groups.

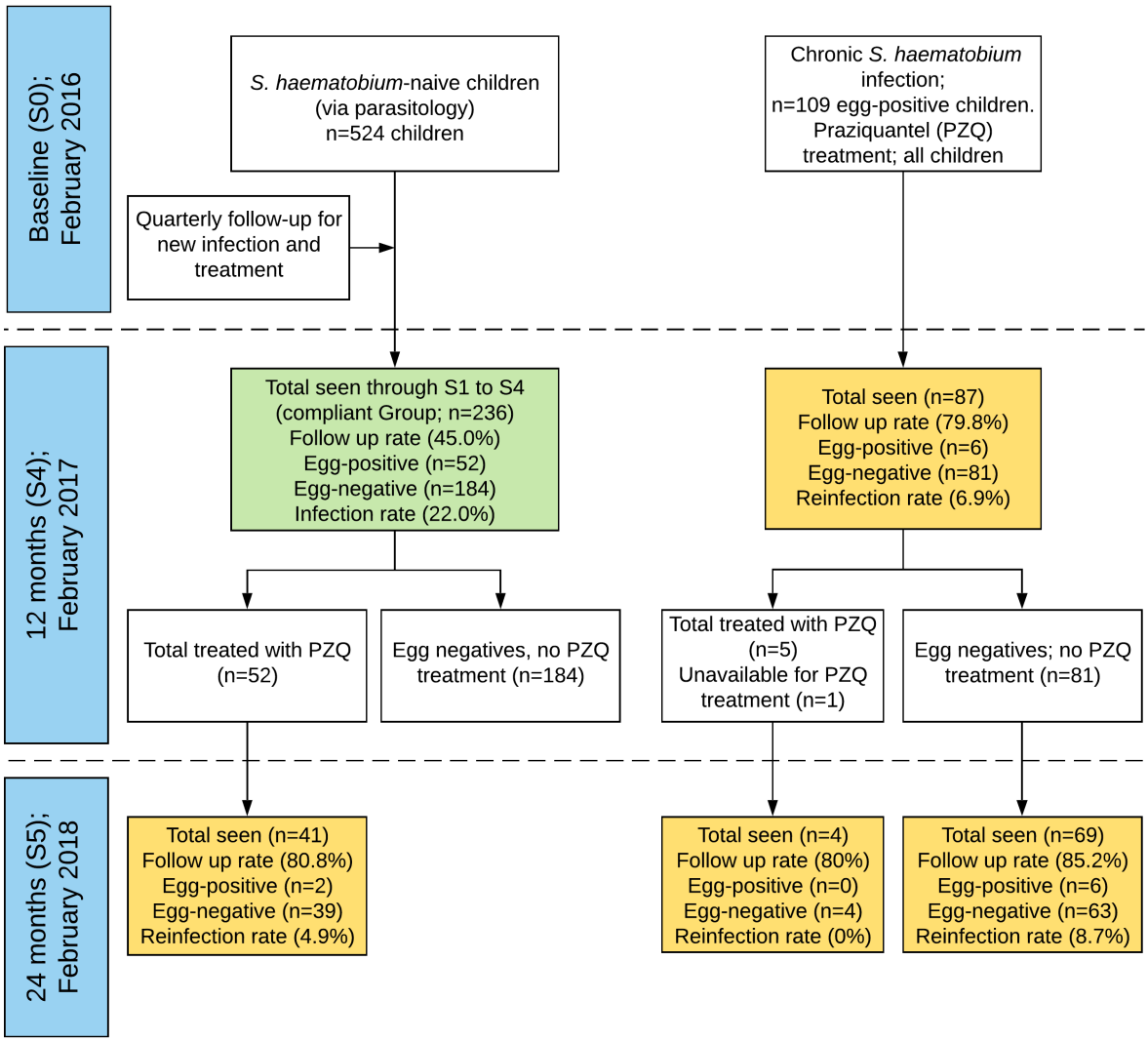

Figure 4.3: Flow chart showing the annual infection and reinfection rates
The number of children (n) and (re)infection rates calculated at each annual time point was limited to the compliant group of children present at each consecutive survey point. Green box= new infection screen; Yellow box= reinfection screen.

4.4.4 Impact of praziquantel treatment on reinfection rates

Figure 4.4 shows the infection and reinfection rates compared between the naive and chronic groups. Compared to baseline, the rate of reinfection at 12 months post-treatment was significantly lower in the chronic group (6.9%, 95% CI= 2.9–14.5, p <0.001), whilst the rate of new infections in the naive group was significantly higher (22.0%, 95% CI= 17.2–27.7, p <0.001). The rate of new infections in the naive group was 3-fold higher than the rate of

reinfections in the previously treated chronic group (6.9%, 95% CI= 2.9–14.5, p= 0.001) [see **Figure 4.4a**].

In the naive group, the reinfection rate at 24 months post-treatment (4.9%, 95% CI= 0.5–17.0) was significantly lower than the rate of new infections at 12 months (22.0%, 95% CI= 17.2–27.7, p= 0.009), as shown in **Figure 4.4b**. This rate of reinfection in the naive group at 24 months (i.e. 4.9%) was similar to that observed within the same time period (i.e. 12 months) at 12 months post-treatment in the chronic group (6.9%, 95% CI= 2.9–14.5, p= 1.000), although slightly lower. In the chronic group, reinfection rates at 24 months post-treatment (8.7%, 95% CI= 3.7–18.0, p= 0.766) was similar to that observed at 12 months post-treatment (**Figure 4.4b**), with no reinfection rates recorded among those who received a second treatment for reinfection at 12 months (see **Figure 4.3**).

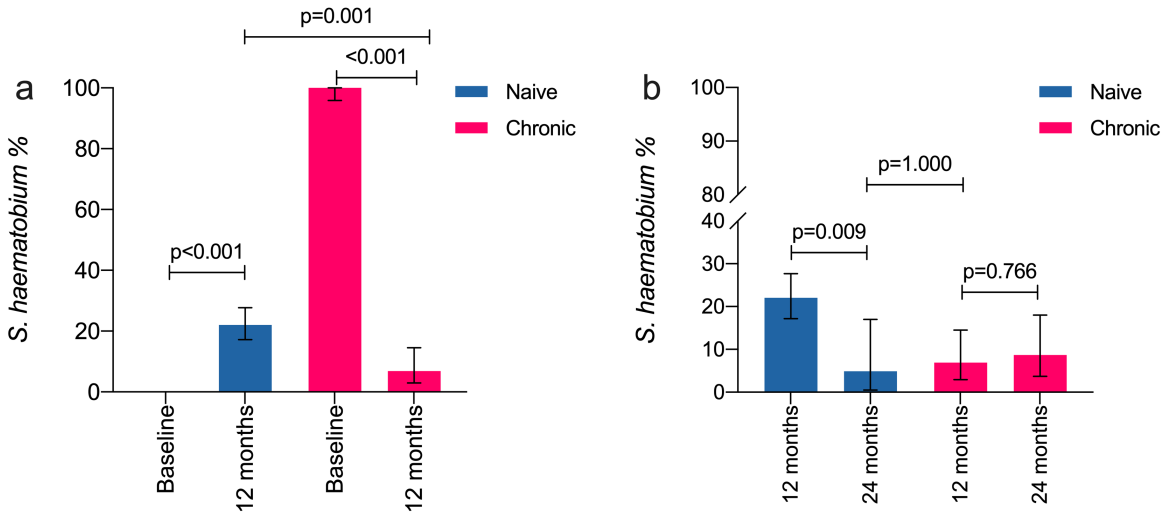

Figure 4.4: Annual *S. haematobium* infection and reinfection rates, stratified by treatment group
a) *S. haematobium* infection and reinfection rates at baseline and at 12 months. For the naive group, the rate of new infections is shown at 12 months, and for the chronic group, infection and reinfection rates are shown at baseline and at 12 months respectively. b) *S. haematobium* infection and reinfection rates at 12 months and at 24 months. For the naive group, the rate of new infections is shown at 12 months and reinfection rate shown at 24 months. For the chronic group, reinfection rates are shown at both survey points. Error bars indicate the 95% confidence intervals. p-values are for Fishers exact tests for comparisons between groups.

4.4.5 Impact of praziquantel treatment on the intensity of reinfection

For the reinfection cohorts only, the mean infection intensity upon reinfection in both the naive and chronic groups were significantly lower, when compared to primary infection intensity levels ($p < 0.001$) [**Figure 4.5**]. The mean infection intensity upon reinfection was similar for both groups ($p = 0.707$), and this was also true for the infection intensity at primary infection ($p = 0.470$).

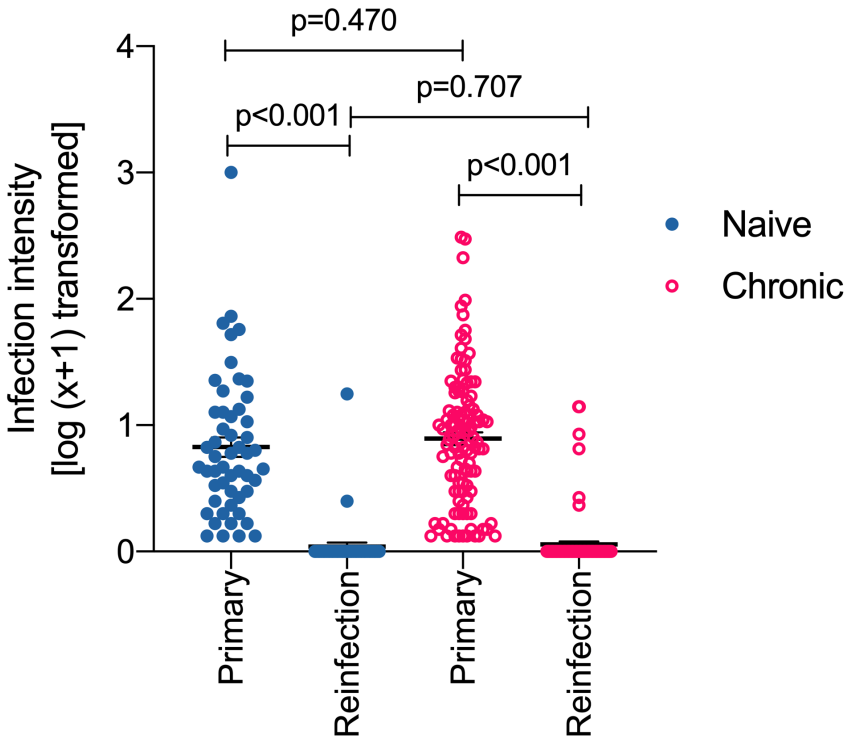

Figure 4.5: *S. haematobium* infection intensity at primary infection and upon reinfection, stratified by treatment group
The scatter plot shows *S. haematobium* infection intensities at both time points and in both groups, limited to the reinfection cohorts only. Infection intensities were transformed [($\log_{10}$ (egg count +1)] to meet normality assumptions for parametric tests. Horizontal lines represent mean (± standard error of the mean). p-values show t-tests for comparisons between groups; paired t-tests for within group comparisons at two time points.

4.5 Discussion

Urogenital schistosomiasis remains a major public health burden among PSAC in tropical and subtropical regions. PZQ treatment for schistosomiasis is effective in PSAC (Mutapi *et al.,* 2011), and has been shown to have an added benefit of inducing/accelerating a protective immune response, which can last for more than a year in older children and adults (Bourke *et al.,* 2013). In this chapter, I determined the impact of a single PZQ treatment on reinfection rates in a cohort of Zimbabwean PSAC experiencing their first schistosome infection. I tested the hypothesis that in previously uninfected children, a single treatment of the first schistosome infection is associated with resistance to reinfection, similar to that observed following treatment of chronic infections, as will occur with MDA treatment strategies. The results showed that in previously uninfected PSAC (naive group), regular quarterly screening and treatment of the first *S. haematobium* infection reduces the actual time at risk of infection in the population, and results in reduced rates of subsequent new infections. PZQ treatment of both the first and chronic schistosome infections, was associated with significantly lower reinfection rates a year later. The rate of reinfection following treatment of first infections was similar to that observed following treatment of chronic infections. The observed effect of treatment on reinfection was commensurate with infection intensity, showing that the infection intensity upon reinfection was significantly lower than the primary infection intensity levels.

In the first year, regular quarterly screening and treatment of first infections in the naive group, resulted in reduced rates of subsequent new infections every quarter. This was reflected in the lower number of child life-years of infection, i.e. the actual time at risk of *S. haematobium* infection, when schistosome infections are detected and treated early in the population. This is consistent with evidence suggesting that antihelminthic treatment of infected children in the population, has the potential of lowering levels of contamination of shared water sources, and subsequently reduces exposure and infection transmission (Hodges *et al.,* 2012, Senghor *et al.,* 2016). It is also plausible that the regular quarterly screening within the community was

accompanied by increased awareness among the caregivers and parents of PSAC, resulting in positive changes to behaviours that put PSAC at risk of schistosome infection (WHO Expert Committee 2002, Beanland *et al.,* 2006).

In the current study, the impact of PZQ treatment on lower reinfection rates and intensity, compared to that of primary infections in both the naive and chronic groups, is consistent with reports from other epidemiological field studies (Wilkins *et al.,* 1987, Wu *et al.,* 1993, N'goran *et al.,* 2001, Kabuyaya *et al.,* 2017). This effect is further confirmed by the fact that within the same year (year 1), the annual rate of first infections in the naive group was about 3-fold higher, when compared to the annual reinfection rate observed in previously treated children from the chronic group. At the time of writing this thesis, the novelty of the current study lies in the fact that in addition to treatment of chronic infections, as will be the case in conventional MDA treatment strategies in endemic areas, my findings show this same effect using a single early treatment in PSAC experiencing their very first schistosome infection. Moreover, the rate of reinfection in the naive group was lower, although not significantly different from that observed in the chronic group. Hence, regular screening to detect and treat schistosome infections early in PSAC will have similar benefits to treating chronic schistosome infections, in terms of resistance to reinfection, if not greater. Residual morbidity or delay in resolution of morbidity post-treatment from chronic schistosome infections is common (Colley *et al.,* 2014). Therefore, as the crux of current treatment and control efforts is to reduce infection intensity and morbidity (Kabatereine *et al.,* 2003), early detection and treatment may have the added benefit of averting long-standing schistosome-related morbidity in PSAC, in addition to reducing reinfection rates.

In the sub-group of children from the chronic group at baseline who were reinfected a year post-treatment, a repeat dose of PZQ treatment was administered, and no reinfection was recorded in the second year. This is in agreement with human field studies in adults, showing

that schistosome-exposed individuals develop resistance to reinfection, with increasing rounds of multiple PZQ treatments (Karanja *et al.,* 2002, Black *et al.,* 2010b).

While the mechanisms underlying the effects of PZQ treatment on reduced reinfection rates and intensity were beyond the scope of the current study, my findings can be contextualised within the current paradigm of the development of acquired protective immunity against schistosomiasis. The findings in the current study are consistent with human immuno-epidemiology studies showing that PZQ treatment of schistosome infections in PSAC (Rujeni *et al.,* 2013), older children, and adults (Watanabe *et al.,* 2007, Black *et al.,* 2010a, Black *et al.,* 2010b, Bourke *et al.,* 2013, Schmiedel *et al.,* 2015), can induce effector responses associated with protection against reinfection. Experimental helminth infection in nematodes has shown that antihelminthic treatment of short-lived primary infections, results in greater levels of treatment-induced acquired protection against reinfection than that achieved by allowing adult worms to reside in the host for several weeks (Behnke and Robinson 1985). This may be relevant to PSAC experiencing their first schistosome infection; screening for and treating infections early may have the added benefit of not allowing infections to persist for longer periods, reducing the possibility of severe morbidity, and the possibility of inducing resistance to reinfection.

This proof-of-principle study clearly demonstrates the health benefits of early treatment of the first schistosome infections in PSAC, in terms of reducing the rate of subsequent new infections, as well as reinfection rates and intensity. However, there is a need for larger studies determining the practical long-term operational and economic implications of regular screening to diagnose and treat primary schistosome infections of all species, early in PSAC. Failure to obtain significant differences in reinfection rates between the naive and chronic groups could be due to the smaller sample sizes of infected individuals followed up for reinfection. In addition, schistosome diagnosis by microscopically enumerating excreted eggs is less sensitive for very low intensity infections, especially in PSAC, thus underestimating the

primary infection rates observed in this study. This may also influence the lower numbers of infected individuals to be followed up for reinfection. Nonetheless, this does not affect the findings in the current study, as the comparison was between pre- and post-treatment infection rates, using the same diagnostic technique and within the same cohorts in each group.

4.6 Conclusions

I have demonstrated that in previously uninfected PSAC, PZQ treatment of the first schistosome infection is associated with a level of resistance to reinfection, similar to that observed following treatment of chronic infections. This effect was commensurate with schistosome infection intensity, showing a significantly lower infection intensity upon reinfection. Regular quarterly screening and treatment of the first *S. haematobium* infection results in reduced rates of subsequent new infections. Given the health consequences in terms of long-term morbidity from chronic schistosome infections, a regular screen-and-treat strategy as part of routine child health and development monitoring activities, will optimise the chances of detecting and treating schistosome infections early, while reducing the risk of new infections, reinfection, as well as severe morbidity.

Chapter 5 Differences in host metabolic profiles in *Schistosoma haematobium* infected vs. uninfected preschool-aged children

5.1 Introduction

Like all other parasites, schistosome worms living inside the definitive human host depend on host resources for survival, and cause it harm (Borgsteede 2004). In endemic areas with high infection transmission, infection with schistosomes is cumulative and can be acquired within the first year of birth (Woolhouse *et al.,* 2000). The health impacts of *Schistosoma haematobium* infection thus begin at this early age and can include haematuria, protein wasting (Wilkins *et al.,* 1979, Colley *et al.,* 2014, Wami *et al.,* 2015, Freer *et al.,* 2018), malnutrition, poor growth, and cognitive impairment (Freer *et al.,* 2018). Left untreated, infection can persist for years, leading to pathology including anaemia, poor reproductive health, increased susceptibility to sexually transmitted infections (Freer *et al.,* 2018), prostate cancer (Tuffour *et al.,* 2018), urothelial carcinogenesis (Gelfand *et al.,* 1967, Ishida and Hsieh 2018), bladder dysfunction, fibrosis, and renal failure (Lichtenberg 1975, Ishida and Hsieh 2018).

Interaction between the schistosome worm and its human host is central for parasite survival and to establish a parasitic relationship. For instance, schistosome worms rely on host glucose for survival (Bueding 1950, Wu *et al.,* 2010b). Schistosome infection also induces parasite-specific T-helper type 2 (Th2) immune responses and host immuno-modulation that promote parasite survival for decades (Pearce and MacDonald 2002, van Riet *et al.,* 2007, Colley and Secor 2014, van der Zande *et al.,* 2019); the changes in host immune responses can alter host metabolic function, disease susceptibility and patterns, and overall host health (Brestoff and Artis 2015). Experimental schistosome studies have shown that in order to establish themselves and survive in the host, schistosome worms require host-derived endocrine steroid and thyroid hormones (de Mendonca *et al.,* 2000, Saule *et al.,* 2002, You *et al.,* 2015), CD4+ T-lymphocytes (Davies *et al.,* 2001), as well as cytokines including tumour necrosis factor

alpha (Amiri *et al.,* 1992, Davies *et al.,* 2004) and interleukin-7 (Saule *et al.,* 2002). When such host factors are unavailable to the parasite, there is poor parasite development and fecundity, combined with reduced pathology in the host (Cheng *et al.,* 2008, Lamb *et al.,* 2010, Tang *et al.,* 2013). Together, the available experimental evidence further indicates that parasite alterations in host pathways extend to metabolic homeostasis. The metabolic phenotypes of mice during *Schistosoma* infection show that infection and disease are linked to alterations in gut microbiota metabolism (Wang *et al.,* 2004, Wu *et al.,* 2010b), as well as changes in amino acid, lipid and energy metabolism (Ahmed and Gad 1995, Wang *et al.,* 2004, Li *et al.,* 2009, Wu *et al.,* 2010b). Alterations in liver metabolism due to parasite egg-induced inflammatory responses have also been reported (Garcia-Perez *et al.,* 2008). Studies characterising the host metabolic phenotype during human schistosomiasis are limited. Nonetheless, two studies (Balog *et al.,* 2011, Panic *et al.,* 2018) have analysed urine samples to characterise the host metabolic changes during human *S. mansoni* infection. The findings reported were similar to those from experimental models, in terms of alterations in energy, liver, and gut microbiota metabolism (Balog *et al.,* 2011, Panic *et al.,* 2018).

While the significant detrimental effects of schistosome infections on host health are unarguable, some experimental and human epidemiological studies have suggested that the sustained host metabolic alterations from schistosome infections may reduce the occurrence and severity of other conditions, including metabolic syndrome (Zaccone *et al.,* 2003, Stanley *et al.,* 2009, Wu *et al.,* 2010b, Chen *et al.,* 2013, Wolde *et al.,* 2019). The exact mechanisms of these effects however remain to be fully understood (van der Zande *et al.,* 2019). There is therefore a need for further studies on the dynamics of host-parasite relations at the molecular level, to elucidate the pathways involved in pathology and disease progression versus those ameliorating metabolic syndromes.

In the case of *S. haematobium* infection in infants and young children, little is known about the basic mechanisms underlying the pathophysiology of the disease, and the disease is poorly

understood at the molecular level (Rollinson 2009). There are limited human studies on the impact of schistosome infection on host metabolism, and metabolic phenotyping of blood samples from cases of human schistosomiasis has not been reported. The majority of previous investigations both in human and in animal models have been based on well-established late stage schistosome infections, not allowing the early metabolic changes associated with the first schistosome infection to be elucidated. Most studies have also focused on *S. mansoni* and *S. japonicum* infections, and there have not yet been any published studies on the impact of human *S. haematobium* infection on the host metabolism.

The aim is of this chapter was to characterise the host metabolite profiles of preschool-aged children (PSAC; aged 5 years and below) before their first *S. haematobium* infection, and the changes that occur following infection and curative treatment. The impact of such metabolite alterations on host metabolic pathways and schistosome-related morbidity was also determined. Using capillary electrophoresis mass spectrometry (CE-MS), the serum metabolite profiles of *S. haematobium*-infected PSAC was determined and compared to that of uninfected children. The findings in this chapter add to the understanding into schistosome infection and its association with the host metabolism, disease progression and morbidity in PSAC. This will inform appropriate new strategies such as nutraceuticals, to reduce schistosome-associated morbidity in control programmes targeted at this age group.

5.2 Hypotheses

(i) *Schistosoma haematobium* infection in PSAC is associated with alterations in host metabolite profiles early in infection.

(ii) The alterations in host metabolite profiles are commensurate with infection intensity, and are linked with host metabolic pathways implicated in schistosome-related morbidity.

5.3 Methods

5.3.1 Study design, population and site

The current study followed a longitudinal study design that included a subset of 83 children (aged 2–5 years), meeting the inclusion criteria for the larger paediatric urogenital schistosomiasis study described in **Chapter 2.** Children not previously infected or treated for schistosomiasis (assessed by health questionnaire and clinical records, as described in **Chapter 2**) were selected from the Madziwa village, and were followed to detect their first *S. haematobium* infection. The group of children included in the current study were selected based on the following: (i) consent from parents/guardians, (ii) availability of socio-demographic data, (iii) availability of parasitology samples (urine and stool samples) for diagnosis at recruitment, (iv) confirmed schistosome negative by *S. haematobium* and *S. mansoni* egg count as part of the baseline survey, and (v) confirmed negative for soil-transmitted helminths (STH). A schematic diagram of the study design is shown in **Figure 5.1.**

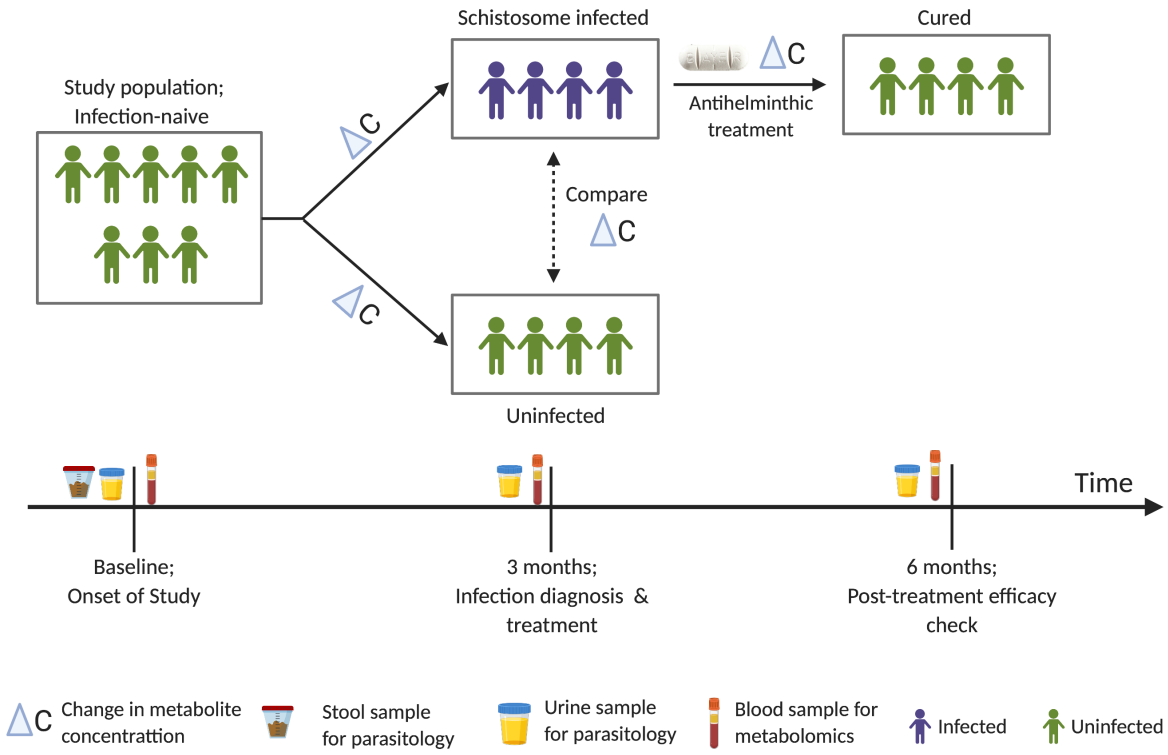

Figure 5.1: Schematic diagram of study design

For the 83 schistosome-negative children recruited at baseline, metadata on socio-demography (including age and sex), and anthropometry (including weight, height, and mid-upper arm circumference) gathered from measurements and questionnaires administered at the time of recruitment were used (details in **Chapter 2**). Growth and nutritional indices (height–for–age Z-scores (HAZ), weight–for–age Z-scores (WAZ) and weight–for–height Z-scores (WHZ)) were calculated from anthropometric measurements, as described in **Chapter 2**. A venous blood sample was also collected from each child and later processed for serum metabolite analysis. Children were then followed up 3 months later to detect their first *S. haematobium* infection. Both experimental and previous field studies show that in 3 months the new *S. haematobium* infections would have reached patency and thus be detectable by egg excretion (Webbe and James 1971, Smith *et al.,* 1976, Wami *et al.,* 2016). At this follow up survey, a second blood sample was collected from all children for serum metabolite analysis to detect changes from baseline levels. All children who were positive for *S. haematobium* infection were treated with a single dose of praziquantel (PZQ) at the standard 40 mg/kg body weight (WHO Expert Committee 2002), as described in **Chapter 2**. For treated children, a post-treatment efficacy check (by egg count) and blood sampling were carried out 3 months later for post-treatment serum metabolite analysis.

5.3.2 Sample collection and processing

Urine and stool samples were collected for parasitological diagnosis of *S. haematobium* infection and to exclude intestinal helminths respectively (*S. mansoni* and STH), as described in **Chapter 2**. Collection of blood samples and further processing for serum were as described in **Chapter 2**. Blood samples were preferred to urine samples for metabolite analysis because it is less susceptible to metabolite variations related to sample collection time and food intake (Kim *et al.,* 2014). Other pre-analytical conditions including avoiding repeated freeze and thaw, and rejection of haemolysed samples were adhered to (Yin *et al.,* 2015). Thus, sample collection and processing conditions followed standard considerations as recommended for

metabolite analysis (Yin *et al.,* 2015). Aliquots of serum samples were shipped on dry ice for sample preparation and metabolite analysis at the Human Metabolome Technologies Inc. (HMT; Yamagata, Japan). Metabolite analysis were carried out by HMT using the capillary electrophoresis time-of-flight mass spectrometry (CE–TOF–MS)-based metabolomics technique, as described in **Chapter 2**.

5.3.3 CE-TOF-MS data acquisition and processing

Peak information including mass-to-charge ratio (*m/z*), migration time (MT), and peak area, were extracted using automatic integration software (MasterHands ver. 2.17.1.11 developed at Keio University) at HMT. Relative peak area was calculated using a peak detection limit based on signal-noise ratio (S/N)= 3 (Soga *et al.,* 2002):

$$Relative\ Peak\ Area = \frac{Metabolite\ Peak\ Area}{Internal\ Standard\ Peak\ Area \times Sample\ Amount} \qquad (5.1)$$

For peak annotation, putative metabolites were then assigned from HMT's standard library and Known–Unknown peak library, based on *m/z* and MT. In instances where a feature matched with multiple annotations within their *m/z* and MT windows, all alternatives are provided. The tolerance level was ±0.5 min in MT and ±10 ppm in *m/z* (Soga *et al.,* 2003):

$$Mass\ error\ (ppm) = \frac{Measured\ Value - Theoretical\ Value}{Measured\ Value} \times 10^6 \qquad (5.2)$$

To quantify target metabolites, single-point (100 μM) calibrations were used to obtain standard curves for each internal metabolite standard added to the sample. Absolute metabolite concentrations were then calculated by normalizing the peak area of each target metabolite with respect to the area of the internal standard. This minimised technical variability in the entire run of the CE–TOF–MS analysis, and enabled sample to sample comparisons in data analysis.

Peak areas of putative metabolites along with concentrations of quantified metabolites, expressed in μM, were received as Excel files from HMT for further analysis. A total of 248 metabolite peaks (145 in cation and 103 in anion mode respectively) were detected and annotated based on HMT's standard and Known–Unknown peak library. Of these, 70 target metabolites were detected and quantified (40 in cation and 30 in anion mode respectively), and these were used for all downstream analysis. Individual samples for which a target peak or metabolite was below detection limits and thus could not be quantified, the peak area or concentration of the metabolite was captured as "Not detected (N.D)".

5.3.4 Data pre-processing

Prior to any downstream analysis, a data integrity check and pre-processing was performed using MetaboAnalyst, a web-based tool for the analysis of metabolomic data (Xia *et al.,* 2009, Xia and Wishart 2011, Chong *et al.,* 2018). First, the entire data set from all three time points was checked for missing values. Overall, metabolite concentration values should be non-negative and without missing values (in this case "N.D"); these cause difficulties in data normalisation and downstream analysis. Since missing or zero values were caused by metabolites with abundance below the detection limit, and not a mere absence, these were replaced by a small pseudo value (i.e. half the minimum positive value in the entire data set= 0.15 μM).

Metabolite data sets were then processed to remove data for metabolites with a constant value across all samples. By default, MetaboAnalyst removes data for metabolites with a constant or a single value across all samples. For example, a metabolite with concentration values of 0.15 μM across all 83 samples will be removed. For the metabolite data set at baseline, six metabolites were found and removed (adenosine triphosphate (ATP), anthranilic acid, betaine aldehyde + H_2O, dihydroxyacetone phosphate, glycerol-3-phosphate, and phosphoenolpyruvic acid).

To determine the change in metabolite concentrations in response to schistosome infection, the change in metabolite concentrations (ΔC) at baseline (C1) and at follow up for infection (C2) was calculated as, ΔC μM= C2 – C1 (μM). The resulting data set was then processed to remove data for metabolites with a constant value across all samples. Likewise, the six metabolites which were found and removed from the baseline metabolite data set were also removed by default. To improve statistical power, eight additional metabolites with less than n= 10 non-zero change in concentration values (ΔC) across all samples were excluded from analysis with the change in concentration metabolite dataset (i.e. 14 metabolites in total excluded; the same six metabolites from baseline analysis, in addition to 2-phosphoglyceric acid, fumaric acid, guanosine diphosphate (GDP), glucose-1-phosphate, glyoxylic acid, guanine, uridine diphosphate (UDP), and uracil).

To allow for biologically-related scaling of data and ensure that all metabolites are treated as equally important during downstream analysis, data were processed by range scaling (mean-centred and divided by the value range of each variable) (van den Berg et al., 2006).

5.3.5 Statistical analyses

Data analyses and visualisation were performed using SPSS version 22 (IBM Corp.), MetaboAnalyst (Xia *et al.,* 2009, Xia and Wishart 2011, Chong *et al.,* 2018), and GraphPad Prism version 8.2.0 (GraphPad Software, Inc). Full details of the statistical methods used here are described in **Chapter 2.** A schematic diagram showing an overview of the statistical methodology used is shown in **Figure 5.2.**

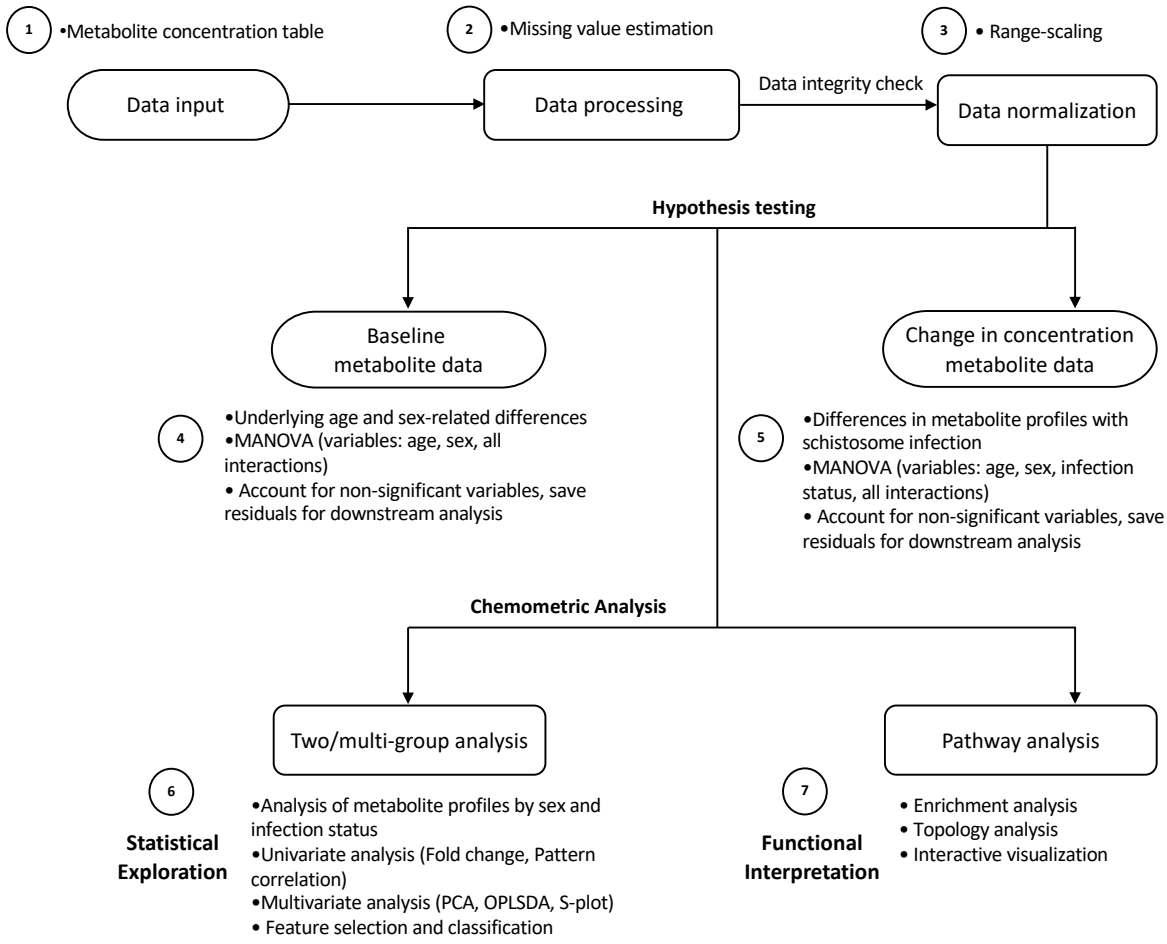

Figure 5.2: Schematic diagram of statistical methodology
MANOVA, Multivariate Analysis of Variance; PCA, Principal Component Analysis; OPLSDA, Orthogonal Projections to Latent Structures Discriminant Analysis

To test the null hypothesis that the mean differences in metabolite profiles between groups of interest are likely due to chance, Multivariate Analysis of Variance (MANOVA; SPSS) with sequential sums of squares was used, as recommended for infectious disease data from human epidemiological studies (Mutapi and Roddam 2002). In this case, the effects of each term are adjusted only for the effects of terms preceding it in the model, and the order in which variables are entered into the model is essential; the variables of interest are thus entered after other confounding variables (Mutapi and Roddam 2002). Where a variable was found to be significant, the model was re-run without the significant variable, and the residuals from the resulting model were saved and subjected to further analysis to identify discriminatory

metabolites compared across that variable (MetaboAnalyst). This was to ensure that the confounding effects of factors such as age and sex were already accounted for, prior to downstream analysis to determine the most relevant metabolites accounting for differences in metabolite profiles between groups of interest.

Age- and sex-dependent effects exist for metabolites (Gu *et al.,* 2009, Fan *et al.,* 2018). Thus, a MANOVA model to determine and account for underlying age and sex-related effects at baseline, which may account for differences in metabolite concentrations post-infection was run. The model included age (years), sex, and their interaction, in that order. To further identify specific metabolites associated with sex at baseline (significant in the initial MANOVA model), the model was re-run with age, and the residuals saved for further analysis in MetaboAnalyst. To determine the change in metabolite concentrations due to schistosome infection, a model including age (years), sex, infection status and their interactions, in that order was used. To further identify metabolites associated with schistosome infection status (significant in the initial MANOVA model), the model was re-run with age, sex, and their interaction, and residuals saved for further analysis.

Residual metabolite concentration data sets (from MANOVA models) along with participant metadata were imported into MetaboAnalyst. Univariate analysis was used to identify metabolites that are potentially different between two groups, and to show metabolite variation patterns under different conditions. Fold change (FC) analysis was used to compare absolute value changes in metabolite concentrations between groups, and a concentration ratio (i.e. between the two groups) of at least 2-fold was considered significant (Patterson *et al.,* 2006). Pattern correlation analysis (Pearson's) was used to determine metabolites showing interesting patterns of change under different conditions, and to look for linear/periodic trends in the dataset. A false discovery rate (FDR) threshold of <0.05 (Benjamini and Hochberg 1995) was considered significant.

Multivariate analysis was then used to identify significant metabolites associated with sex and schistosome infection. For an informative first-hand look at the dataset, an unsupervised Principal Component Analysis (PCA) was used to assess clustering trends and group separation in the dataset. To identify the specific metabolites accounting for differences in metabolite profiles between groups of interest, a supervised multiple regression analysis method, Orthogonal Projections to Latent Structures Discriminant Analysis (OPLSDA) (Wiklund *et al.,* 2008) with Orthogonal Signal Correction (OSC) filtering (Wold *et al.,* 1998), was used to discriminate groups and identify the differentially expressed metabolites that drive group separation. This supervised method has been shown to be more reliable at overcoming the limitations of heterogeneity associated with analysis of human metabolomic data, where PCA does not reveal changes in metabolite profiles across groups (Singer *et al.,* 2007, Balog *et al.,* 2011). Cumulative model statistics, $R^2Y(cum)$ and $Q^2(cum)$, were calculated for each model and used to assess the degree of fit and predictive reliability respectively (Wold *et al.,* 2001). The significance of the model was evaluated using permutation testing (n= 1000), with a p-value threshold of <0.05 (Szymanska *et al.,* 2012).

5.3.5.1 Model selection of significant metabolites

For all valid OPLSDA models, an S-plot showing the variable importance in a model, combining the covariance or contributions [X-axis; p(1)] and the correlation or reliability coefficient [Y-axis; p(corr)] loading profile was generated. This was used to identify and select significant metabolites with the highest correlation coefficient within groups (p(corr)) and with the highest contribution to the model separation between groups (p(1)). The p(corr) values are robust to variable selection in the OPLSDA model and are thus comparable between different models (Wheelock and Wheelock 2013). The variable importance in the projection (VIP), a weighted sum of squares of the partial least square (PLS) loadings, taking into account the amount of explained group variation in each dimension, was also calculated for each component. As recommended, a combination of the S-plot [cut of: absolute p(corr) >0.5] and

a VIP value (cut off: VIP ≥1.5) was used to identify and select significant differentially expressed metabolites between groups (Wheelock and Wheelock 2013).

5.3.5.2 Relationship with infection burden

To further determine the relationship between significant differentially expressed metabolites during *S. haematobium* infection and infection burden, range-scaled residuals from the change in concentration metabolite data set (from MANOVA models) were regressed on the log-transformed infection intensity (log_{10} [egg count+1]).

5.3.5.3 Pathway enrichment analysis and topology analysis

To identify metabolic pathways associated with schistosome infection and facilitate further biological interpretation of the metabolite alterations observed, metabolite pathway analysis was performed using MetaboAnalyst. Data for the differentially expressed metabolites identified from the infection status OPLSDA model were queried against associated *Homo sapien*s metabolic pathway libraries (downloaded on 04.06.2019), curated from Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/). The pathway analysis combines results from powerful pathway enrichment analysis with pathway topology analysis to identify the most significant pathways.

The pathway enrichment analysis entailed quantitative enrichment analysis, using the metabolite concentration values to identify subtle but consistent changes amongst metabolites involved in the same biological pathway. As MetaboAnalyst is a web-based tool, the Global Test was used and p-values were approximated based on the asymptotic distribution without using permutations; this is suitable when the most relevant pathways are to be identified, and thus the rank of the pathway is most essential. The Global Test allows the use of metabolites selected based on prior analysis, and to investigate groups of differentially expressed metabolites of biological interest (Goeman *et al.,* 2004). The pathway topology analysis in MetaboAnalyst takes into consideration the structure of biological pathways to estimate significant pathways that change under different conditions. The method of out-degree

centrality, which represents the number of links that are initiated from a node (metabolite), was used. Under this method, it is assumed that nodes upstream will have regulatory roles for the downstream nodes, not vice versa (i.e. assuming that upstream metabolites will have regulatory effects on downstream metabolites but not vice versa, and that changes in more important positions of a network will trigger a more severe impact) (Aittokallio and Schwikowski 2006).

5.4 Results

5.4.1 Population characteristics

As shown in **Table 5.1**, median age was 3 years (range; 2–5 years) and 41 (49.4%) of the children were female. At follow up, 11 (13.3%) were positive for *S. haematobium* infection with mean infection intensity of 0.8 (SEM= 0.3; 95% CI = 0.2–1.5) eggs/10 mL of urine.

Table 5.1: Participant characteristics

Variable	Total	Female	Male
	Baseline		
Age (years)			
Median	3 (3-4)	3 (3-4)	3.5 (3-4.3)
2	8 (9.6)	4 (9.8)	4 (9.5)
3	35 (42.2)	18 (43.9)	17 (40.5)
4	25 (30.1)	14 (34.1)	11 (26.2)
5	15 (18.1)	5 (12.2)	10 (23.8)
Height (cm)	96.0 (91.0-102.0)	96.0 (91.5-98.0)	100.0 (91.0-104.0)
Weight (kg)	14.0 (12.7-16.0)	14.0 (12.1-15.0)	15.0 (13.0-16.0)
MUAC (cm)	15.0 (14.0-16.0)	14.6 (14.0-15.4)	15.0 (14.0-16.0)
Mean WHZ	-0.29 (1.29)	-0.30 (1.23)	-0.28 (1.36)
Mean WAZ	-0.59 (1.19)	-0.62 (1.23)	-0.56 (1.16)
Mean HAZ	-0.70 (1.38)	-0.69 (1.45)	-0.71 (1.33)
	Follow up (3 months)		
***S. h* Infection status**			
Negative	72 (86.7)	34 (82.9)	38 (90.5)
Positive	11 (13.3)	7 (17.1)	4 (9.5)
***S. h* Infection intensity (eggs/10 ml urine)**	0.8 (0.2–1.5)	0.5 (0.1–1.0)	1.1 (0–2.3)

The table shows characteristics of the sample population at baseline and at follow up. Growth and nutritional indices adjusted for age and expressed as Z-scores (Mondal *et al.,* 2012), were calculated using the WHO Anthro software (version 3.0.1), as previously described in **Chapter 2**. Data are expressed as median (IQR) or n (%), except for WHZ, WAZ, and HAZ, which are mean (SD). *S. haematobium* infection intensity is shown as mean (95% confidence interval). *S. h*., *S. haematobium*; WHZ, weight–for-height Z-scores; WAZ, weight–for-age Z-scores HAZ, height-for-age Z-scores.

5.4.2 Metabolic differences associated with age and sex

Initial MANOVA models were built using baseline metabolite profiles to identify any underlying age and sex metabolite variations in the study population, pre-infection, and to identify any potential confounders. From the baseline model, metabolites were found to vary across sex, but not age. The model was then re-run with age only, and the residuals were used to identify the specific metabolites that vary with sex (**Table 5.2**).

Table 5.2: Baseline MANOVA output for age and sex influence on metabolite data

Variable	F value	Hypothesis/Total df	Error df	Partial Eta-squared	p-value
Intercept	0	64	16	0	1.000
Age	1.259	64	16	0.834	0.314
Sex	2.736	64	16	0.916	0.014
Age * Sex	0.619	64	16	0.712	0.910
Equation: Intercept + Age + Sex +Age * Sex					
	Model to obtain residuals				
Intercept	1.066	64	18	0.791	0.462
Age	1.133	64	18	0.801	0.400
Equation: Intercept + Age					

df, degrees of freedom.

As shown in **Figure 5.3**, univariate FC analysis showed that the concentrations of 16 metabolites were lower/down-regulated (≥2-fold) in females (panel a). Pattern correlation analysis showed that metabolite concentrations tend to be higher in males (panel b); of these, 7 metabolites showing this pattern were statistically significant. This included creatinine ($p < 0.001$), citrulline ($p < 0.001$), cis-asconitic acid ($p = 0.004$), gamma-aminobutyric acid (GABA; $p = 0.006$), sarcosine ($p = 0.014$), 2-oxoisovaleric acid ($p = 0.040$), and isocitric acid ($p = 0.040$). After an FDR correction, creatinine (FDR <0.001) and citrulline (FDR <0.001) remained significant. The output details from FC and pattern correlation analysis are shown in **Supplementary Tables 1 and 2 in Appendix B**.

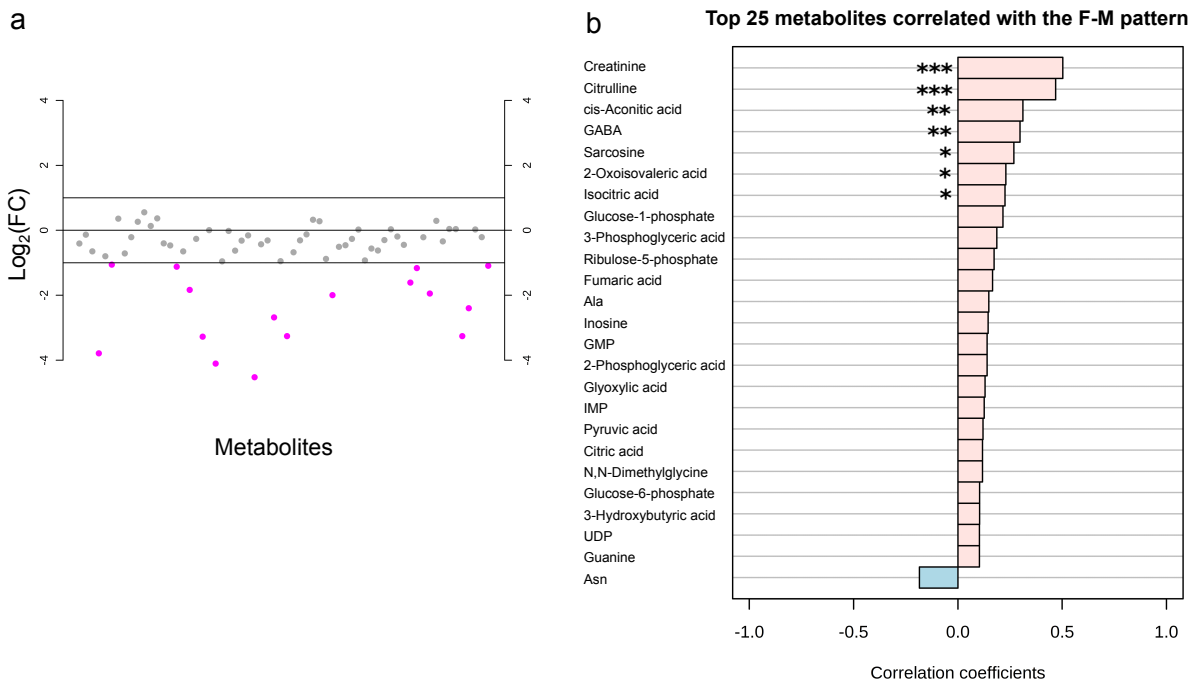

Figure 5.3: Metabolite profiles by sex (univariate)
a) Metabolites identified by fold change (FC) analysis of female/male ratio with threshold of 2-FC. Values are on a log scale to show both up-regulated and down-regulated metabolites symmetrically. The plot shows metabolites that are up-regulated (positive-log scale) or down-regulated (negative log-scale). Pink symbols represent metabolites above the 2-FC threshold. b) Pattern correlation analysis showing metabolites (based on unadjusted p-values), showing different patterns between female and male. GABA, gamma-aminobutyric acid; Ala, alanine; GMP, guanosine monophosphate; IMP, inosine monophosphate; UDP, uridine diphosphate; Asn, asparagine; F, female (n=41); M, male (n=42). ***, p <0.001; **, p <0.01; *, p <0.05.

Based on the univariate analysis, multivariate analysis was used to determine significant metabolites associated with sex. Initial PCA analysis with a model of five components explaining 64.2% of the variability, was aimed at identifying clustering according to sex. The PCA model however did not show any clear clustering by sex (**Supplementary Figure 1 in Appendix B**). This reflects the heterogeneity of data associated with human metabolomic studies (Singer *et al.,* 2007), in contrast to animal models where in-bred animals may contribute to clustering within the first few components (Wang *et al.,* 2004). To unmask changes, a supervised OPLSDA model was used to identify significant metabolites associated with sex (N= 83, 1 predictive and 1 orthogonal component; Q^2= 0.146, p= 0.001 and R^2Y= 0.352, p= 0.033; validation plots are shown in **Supplementary Figure 2 in**

Appendix B), from which a coefficient S-plot was used to identify significantly contributing metabolites discriminating between male and female. Based on the set selection criteria, males were observed to have increased concentrations of creatinine (p(corr)= 0.8, VIP= 2.9), citrulline (p(corr)= 0.7, VIP= 3.1), sarcosine (p(corr)= 0.6, VIP= 1.7), and GABA (p(corr)= 0.5, VIP= 2.4) compared to females (**Figure 5.4**; output details in **Supplementary Table 3 in Appendix B**).

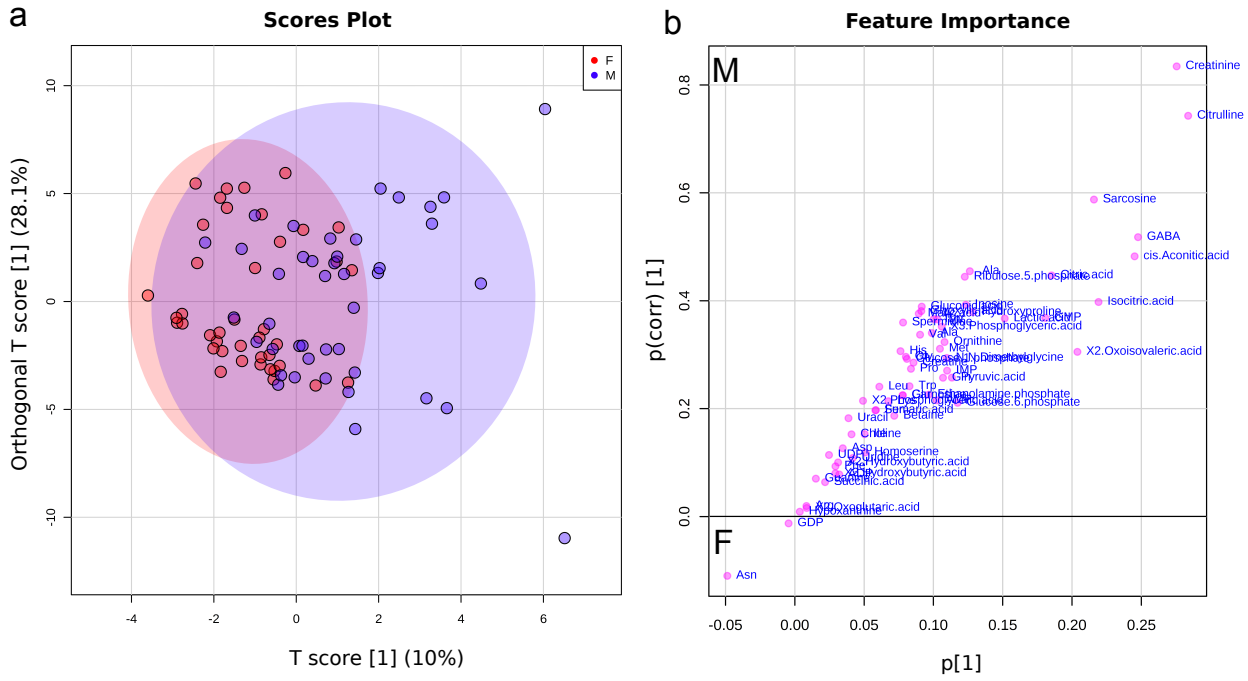

Figure 5.4: Metabolite profiles by sex (multivariate)
a) Score plot and b) Coefficient S-plot based on OPLSDA model of metabolite distribution according to sex (Y variable). Metabolites that significantly influenced the model (absolute p(corr) >0.5 and VIP ≥1.5) were creatinine, citrulline, sarcosine and GABA (gamma-aminobutyric acid). For the S-plot, the y-axis represents the correlation or reliability coefficient and the x–axis represents the covariance or contributions of each metabolite to the model with respect to sex. F, female (n=41); M, male (n=42).

5.4.3 Metabolic profiles during early schistosome infection

Due to the baseline variations in metabolite concentrations related to sex, subsequent models to determine change in metabolite concentrations with schistosome infection were verified to account for potential bias. MANOVA models were built on change in metabolite concentration profiles to determine any associations with *S. haematobium* infection, accounting for age and

sex. Results showed that *S. haematobium* status was associated with metabolite concentrations. For further downstream analysis, the model was re-run with age and sex only, and the residuals were used to identify metabolite features that vary by *S. haematobium* infection status (see **Table 5.3** below).

Table 5.3: Follow-up MANOVA output for influence of infection on metabolite data

Variable	F value	Hypothesis/Total df	Error df	Partial Eta-squared	p-value
Intercept	0	56	21	0	1.000
Age	1.432	56	21	0.792	0.184
Sex	0.742	56	21	0.664	0.813
Infection status	2.231	56	21	0.856	0.023
Age * Sex	0.960	56	21	0.719	0.567
Sex * Infection status	1.901	56	21	0.835	0.053
Age * Infection status	1.056	56	21	0.738	0.463
Equation: Intercept + Age + Sex + Infection status+ Age * Sex + Sex * Infection status + Age * Infection status					
	Model to obtain residuals				
Intercept	0	56	24	0	1.000
Age	1.214	56	24	0.739	0.307
Sex	0.778	56	24	0.645	0.782
Age * Sex	1.002	56	24	0.700	0.516
Equation: Intercept + Age + Sex +Age * Sex					

df, degrees of freedom.

Univariate FC analysis showed that 25 metabolites were either up-regulated or down-regulated in schistosome infection (>2-fold) (**Figure 5.5a**). Based on pattern correlation analysis, different metabolites showed either an increasing or decreasing pattern with schistosome infection (**Figure 5.5b)**; of these, 16 metabolites showing this pattern were statistically significant. Metabolites that showed a pattern of increasing concentration with schistosome infection included adenosine diphosphate (ADP; p <0.001), 3-phosphoglyceric acid (3-PG; p <0.001), adenosine monophosphate (AMP; p <0.001), inosine (p= 0.012), asparagine (p= 0.016), 2-hydroxybutyric acid (p= 0.021), sarcosine (p= 0.023), guanosine monophosphate (GMP; p= 0.032); glucose-6-phosphate (G6P; p= 0.043), and ethanolamine phosphate (p= 0.043). Metabolites that showed a pattern of decreasing concentration with schistosome

infection included lactic acid (p= 0.017), choline (p= 0.017), serine (p= 0.020), cis-asconitic acid (p= 0.023), histidine (p= 0.031), and glutamic acid (p= 0.045). After an FDR correction, ADP (FDR= 0.003), 3-PG (FDR= 0.003), and AMP (FDR= 0.014) remained significant. The output details from FC and pattern correlation analysis are shown in **Supplementary Tables 4 and 5 in Appendix B**).

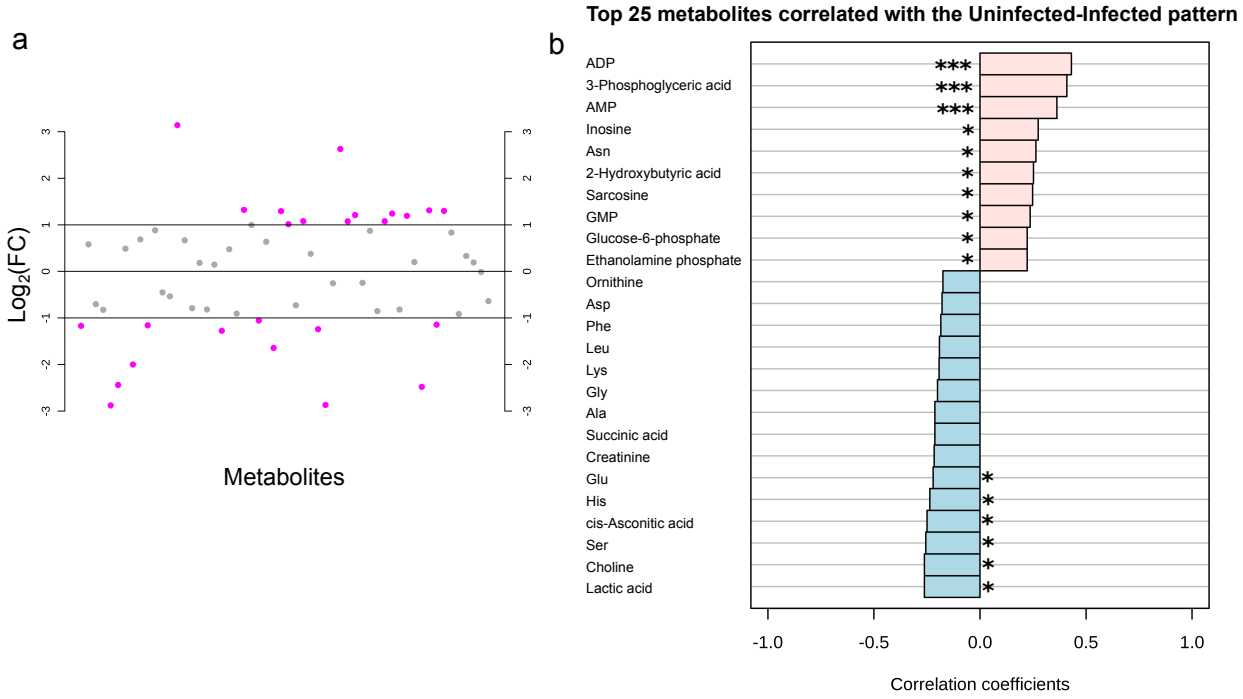

Figure 5.5: Metabolite profiles by schistosome infection status (univariate)
a) Metabolites identified by fold change (FC) analysis by uninfected/infected ratio with threshold of 2-FC. Values are on a log scale to show both up-regulated and down-regulated metabolites symmetrically. The plot shows metabolites that are up-regulated (positive-log scale) or down-regulated (negative log-scale). Pink symbols represent metabolites above the 2-FC threshold. b) Pattern correlation analysis showing the top 25 metabolites (based on unadjusted p-values), showing different patterns between schistosome negative (n=72) and positive (n=11) children. ADP, adenosine diphosphate; AMP, adenosine monophosphate; Asn: asparagine; GMP, guanosine monophosphate; Asp; aspartic acid, Phe, phenylalanine; Leu, leucine; Lys, lysine; Gly, glycine; Ala, alanine; Glu, glutamic acid; His, histidine; Ser, serine. ***, p <0.001; **, p <0.01; *, p <0.05.

Multivariate analysis was used to determine significant metabolites associated with schistosome infection. Initial PCA analysis with a model of five components explaining 57.5% of the variability, was used to identify clustering according to infection status. Likewise, the PCA model did not show any clear clustering by infection status (**Supplementary Figure 3 in Appendix B**), and heterogeneity in the data set may conceal metabolic changes characteristic of infection within the first few components (Singer *et al.,* 2007). To unmask such changes, a supervised OPLSDA model was used (N= 83, 1 predictive and 1 orthogonal component; Q^2= 0.197, p= 0.001 and R^2Y= 0.465, p= 0.001; validation plots are shown in **Supplementary Figure 4 in Appendix B**), from which a coefficient S-plot was used to identify significant metabolites discriminating between infected and uninfected children. Based on the selection criteria, *S. haematobium*-infected children were found to have increased concentrations of AMP (p(corr)= 0.7, VIP= 1.6), 3-PG (p(corr)= 0.7, VIP= 1.5), ADP (p(corr)= 0.6, VIP= 2.3), and G6P (p(corr)= 0.6, VIP= 1.5) [**Figure 5.6**; details in **Supplementary Table 6 in Appendix B**].

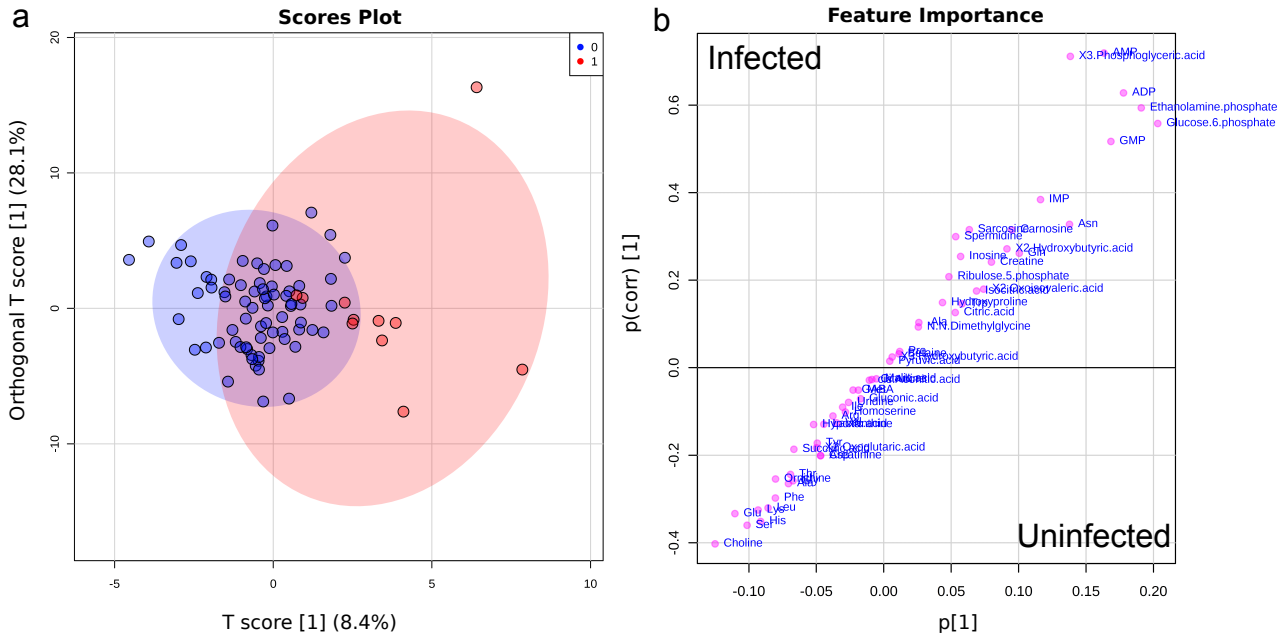

Figure 5.6: Metabolite profiles by schistosome infection status (multivariate)
a) Score plot and b) coefficient S-plot based on OPLSDA model of metabolite distribution according to infection status (Y variable). Metabolites that significantly influenced the model (absolute p(corr) >0.5 and VIP ≥1.5) were adenosine monophosphate (AMP), 3-phosphoglyceric acid (3-PG), adenosine diphosphate (ADP), and glucose-6-phosphate (G6P). For the S-plot, the y-axis represents the correlation or reliability coefficient and the x–axis represents the covariance or contributions of each metabolite to the model with respect to infection status. Infection status was coded as 0 and 1 for uninfected (n=72) and infected (n=11) respectively.

Of interest, a MANOVA model to determine the influence of infection intensity on the significant metabolites identified by OPLSDA (accounted for age and sex), was significant (F-value= 5.178, Partial Eta-squared= 0.21, p= 0.001). As shown in **Figure 5.7**, concentrations of all observed metabolites associated with schistosome infection, increased as infection intensity increased. This relationship was significant for all metabolites, except G6P.

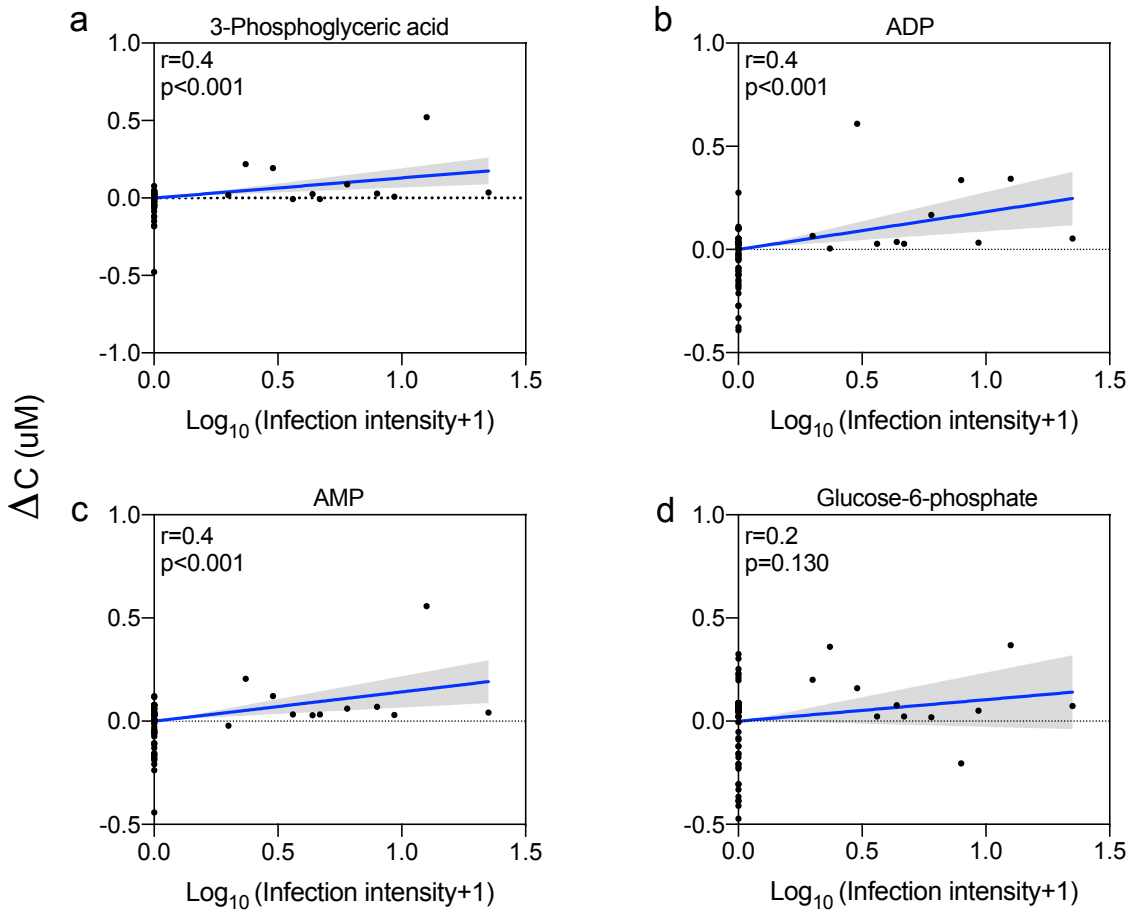

Figure 5.7: Observed metabolite changes are commensurate with schistosome infection intensity. Figures a–d) Scatter plots showing linear regression analysis of infection intensity and change in metabolite concentration (ΔC) of the specific metabolites identified by OPLSDA as associated with *S. haematobium* infection (n=83 independent samples). *S. haematobium* infection intensity was log-transformed [$\log_{10}$ (egg count+1)]. Shaded areas indicate the 95% confidence intervals. AMP, adenosine monophosphate; ADP, adenosine diphosphate.

5.4.4 Effects of curative treatment on metabolite profiles

Of the 11 *S. haematobium*-positive children, a post-treatment follow-up sampling was conducted 3 months later to determine treatment efficacy, with a follow up rate of 6/11 (54.5%). Cure rate and egg reduction rates (as estimated by egg counts) were 100% in the six children followed up, and the impact of treatment on the concentration of the observed metabolites was determined in these six children. As shown in the heat map below, pattern analysis across all three time points showed that metabolite concentrations increased at infection and reduced to near pre-infection levels, post-treatment. Although not significant

(p >0.05), pattern correlation analysis showed that metabolite features exhibit a decreasing trend from infection to when children are cured (**Figure 5.8**; output details in **Supplementary Table 7 in Appendix B**).

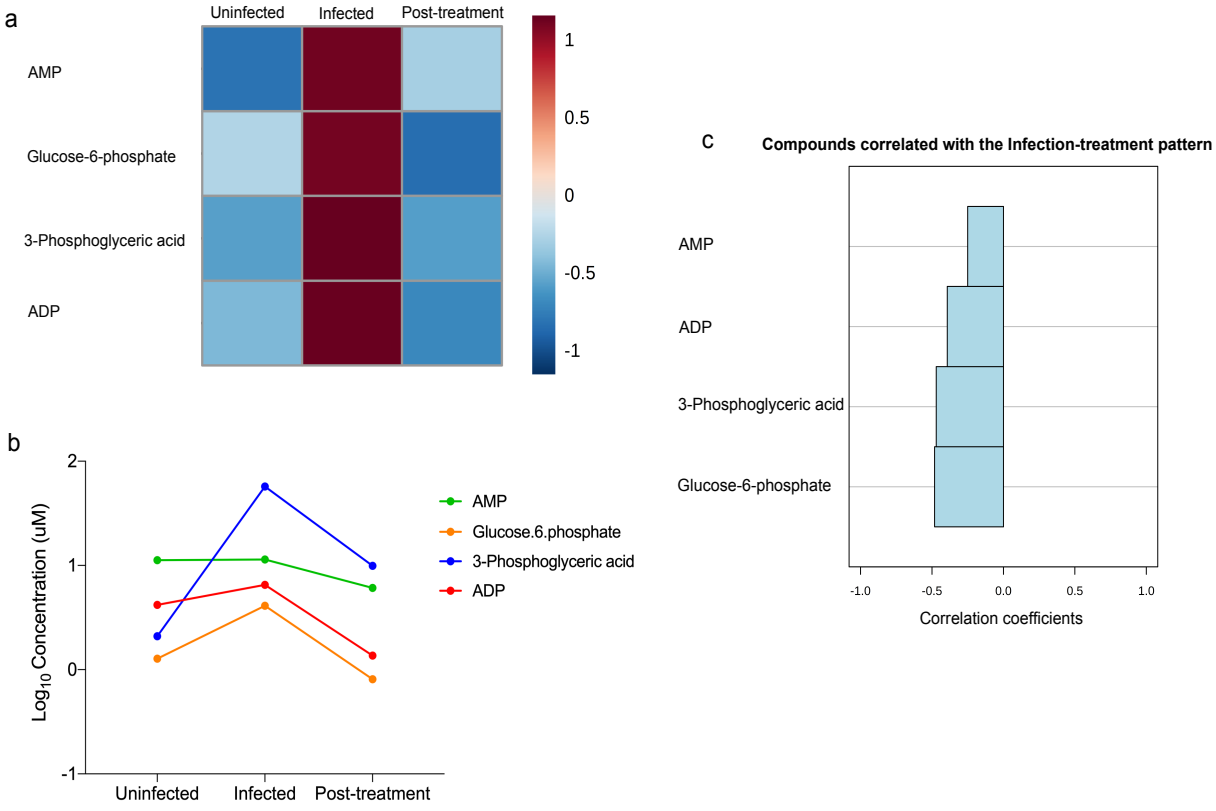

Figure 5.8: Metabolite concentrations decrease to near-uninfected levels after treatment of infection

a) Heatmap showing the mean concentration patterns of specific metabolites (associated with *S. haematobium* infection) at pre-infection, infection and at post-treatment. Colour scale (1 to -1) shows Pearson's correlation co-efficient for up-regulated (positive scale) and down-regulated (negative scale) metabolites. b) Plot shows the absolute mean concentration of metabolites (associated with *S. haematobium* infection) at pre-infection, infection and at post-treatment. c) Pattern correlation analysis showing decreasing patterns from schistosome positive to negative at post-treatment (increasing order of absolute correlation co-efficient). ADP, adenosine diphosphate; AMP, adenosine monophosphate.

5.4.5 Metabolite pathways associated with schistosome infection

The metabolite pathway analysis assigned metabolite compounds in a total of seven pathways, which were identified together as important for the host response to schistosome infection (the full individual metabolic pathways are shown in **Supplementary Figures 5 and 6 in Appendix B**). The predominant hits were energy and purine pathways involved in glycolysis or gluconeogenesis, purine metabolism, pentose phosphate pathway (PPP), starch and sucrose metabolism, galactose metabolism, amino acid and nucleotide sugars metabolism, and nitrogen metabolism in order of decreasing impact and significance (**Figure 5.9**; output details in **Supplementary Table 8 in Appendix B**; the statistical p-values from enrichment analysis are further adjusted for multiple pathway testing).

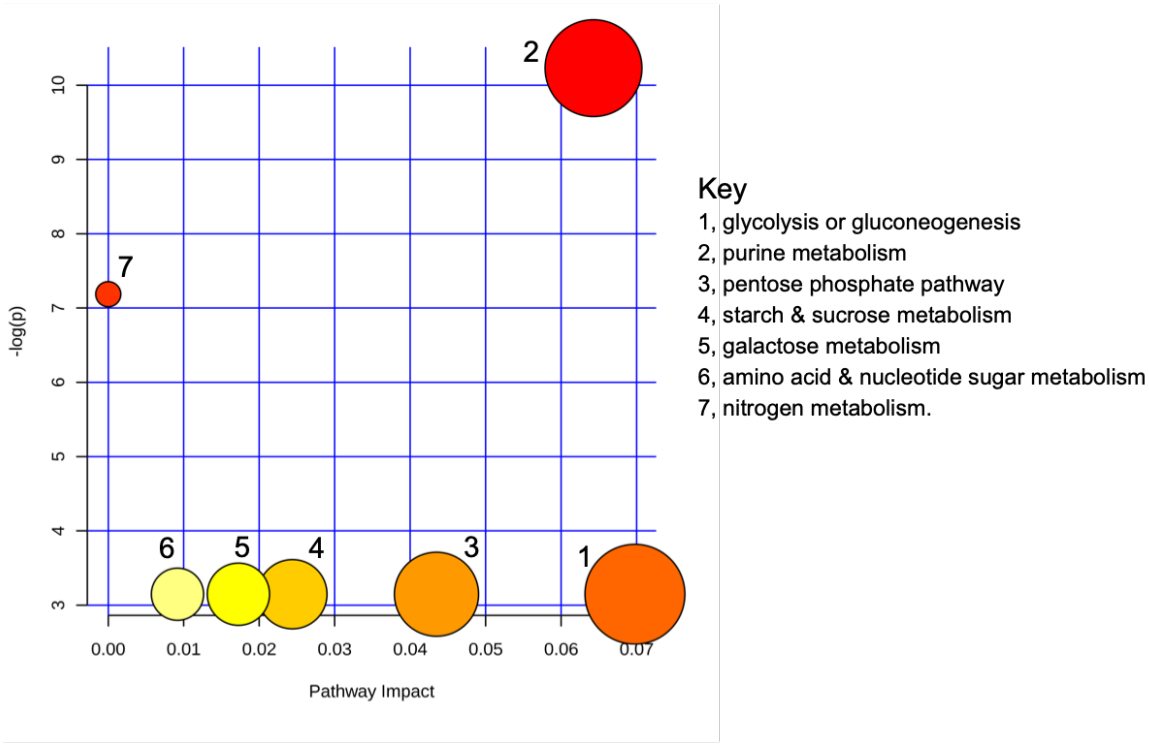

Figure 5.9: Summary of metabolite pathway analysis
Pathway map showing the affected metabolic pathways. The map was generated in MetaboAnalyst and shows all matched pathways according to the p-values from the pathway enrichment analysis, and pathway impact values from the pathway topology analysis. The size of each circle represents the degree of impact on the pathway, and significance levels based on p-values range from yellow (least significant) to red (most significant).

Based on the pathway analysis, two biological hypotheses were proposed for the metabolite alterations observed (**Figure 5.10**). The adult worm uses large amounts of host glucose and energy (Bueding 1950). This stimulates host glycolysis and leads to an increase in host ADP and AMP (Carling *et al.,* 2012, Hardie *et al.,* 2012). Energy demand for increased protein synthesis could also lead to direct dephosphorylation of ATP, increasing host AMP. Energy demands from the parasite also increases host glycogenolysis, gluconeogenesis and fatty acid synthesis, increasing the levels of host G6P and 3-PG, essential to enhance the pentose phosphate pathway (PPP) and fatty acid synthesis respectively. In addition, schistosome tegumental phosphatases and phosphohydrolases e.g. SmAP (Cesari *et al.,* 1981, Bhardwaj and Skelly 2011), dephosphorylate the increased exogenous host phosphate molecules (G6P, AMP, ADP), for parasite purine (i.e. adenosine) and glucose uptake. The resulting extracellular adenosine is also known to dampen host immunity (Hasko and Cronstein 2004, Bours *et al.,* 2006, Gessi *et al.,* 2007) and induce host immunomodulation (van Riet *et al.,* 2007) essential for parasite survival.

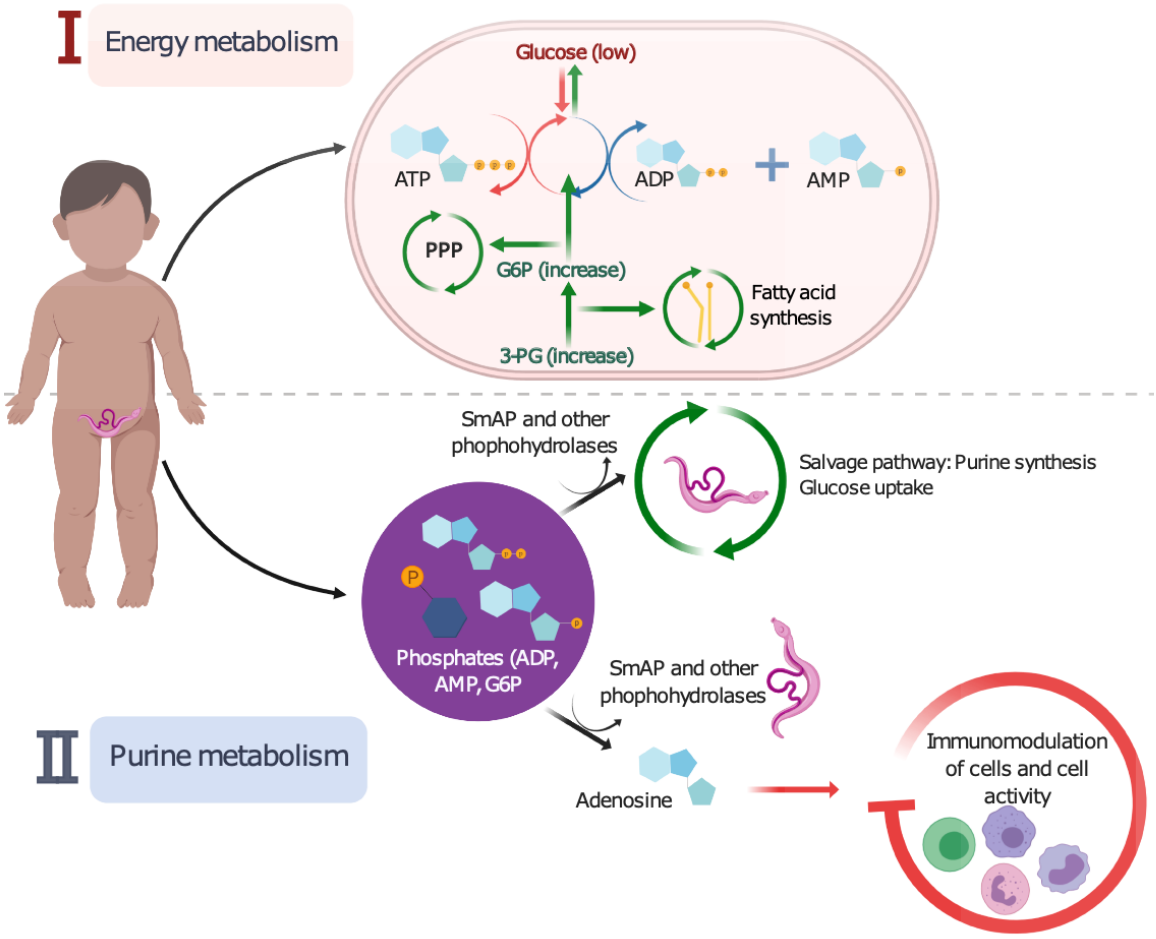

Figure 5.10: Pathway-based model related to the observed metabolic alterations in early *S. haematobium* infection
In response to schistosome infection and increased demands from the host, there is ↑ glycolysis, ↑ pentose phosphate pathway, ↑ fatty acid synthesis ↑ gluconeogenesis ↑glycogenolysis, as well as ↑ purine synthesis for salvage by the parasite, leading to the observed increases in the host metabolites identified (i.e. AMP, ADP, 3-PG, and G6P). AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; 3-PG, 3-phosphoglyceric acid; PPP, pentose phosphate pathway; SmAP, schistosome tegumental alkaline phosphatase.

5.5 Discussion

Host-parasite interactions during schistosome infection are underpinned by the exchange of essential metabolites between the host and the parasite (as described above in **section 5.1**). Metabolomic studies thus help to provide understanding of the host metabolic responses to infection. Unlike animal studies, the health and ethical challenges of conducting schistosome infection and mechanistic studies in humans (as discussed in **Chapter 2**) have limited our

understanding into infection and disease mechanisms relating to the aetiology, as well as clinical manifestations of morbidity and immunopathology. In experimental studies, it is possible to infect animals at specific time points, follow the course of infection, and investigate its impact on the host; this is however not possible in human hosts. To overcome this challenge, the current study followed *S. haematobium* infection of PSAC living in a schistosome-endemic area in Zimbabwe, using a natural infection time-course design. A cohort of PSAC for whom there was no evidence of previous infection by schistosomes, was followed to their first schistosome infection and curative treatment. This was informed by knowledge of exposure to infection, and from previous sero-epidemiology studies in PSAC conducted by our research group (Rujeni *et al.,* 2013, Wami *et al.,* 2014). Using a comprehensive mass spectrometry-based approach, the results demonstrate that the first *S. haematobium* infection is associated with alterations in host metabolites, primarily linked with energy and purine metabolism. The observed changes were commensurate with increasing infection intensity, a confirmation that the changes were associated with the presence of *S. haematobium* infection. Metabolite levels were restored to almost pre-infection levels, following curative treatment with the antihelminthic drug, PZQ.

The metabolic profiles of children were analysed at baseline in order to characterise metabolites in the absence of schistosome infection, as well as to account for confounding factors in subsequent analysis for metabolite changes upon schistosome infection. Levels of creatinine, citrulline, sarcosine/*N*-methylglycine and GABA were higher in males than in females. A potential explanation for this difference in amino acid metabolites could be differences in weight between the children. In adults, increased amino acid metabolites have been attributed to higher muscle mass in males (Lukaski 1996), and this is consistent with the higher weight and associated weight–for-age Z-scores (a standardised assessment of weight in young children, relative to age (MOH Malawi 2016)) in the male children included in the current study. However, there are limited studies on protein and amino acid metabolism in

healthy children, and there is a need for further studies to determine if the observations in adults translate to body profiles in young children (Markofski and Volpi 2011).

At follow-up within 3 months of first schistosome infection, there were significant increases in AMP, ADP, 3-PG, and G6P, compared to uninfected children, and these increases correlated positively with infection intensity. Metabolic pathway analysis showed that the increases were related predominantly to energy (glycolysis, PPP, starch, and galactose) and purine metabolism. This is consistent with findings from studies conducted in experimental models of schistosome infection (Ahmed and Gad 1995, Wang *et al.,* 2004, Liu *et al.,* 2019), showing that such host metabolic alterations can begin as early as three weeks post-infection (Wu *et al.,* 2010b).

The observed increases in discriminatory metabolites associated with schistosome infection has a physiological explanation. *S. haematobium* predominantly resides in the venous plexus of the bladder with direct access to the flow of nutrients in blood. Experimental studies have shown that the schistosome worm relies on the host's glucose for survival (Wu *et al.,* 2010b). Studies have long established that every 5–6 hours, schistosome parasites utilise their dry body weight's worth of glucose from the host, marked by increased lactate and reduced glucose levels in the host blood stream (Bueding 1950, Schiller *et al.,* 1975, Bueding and Fisher 1982). Another consequence of schistosome infection is liver injury, as confirmed by histology in experimental studies (Wu *et al.,* 2010b) and in enzymatic human studies (Abdel-Rahim *et al.,* 1990). The liver injury is marked by stimulated host glycolysis, manifested by reduced plasma glucose as well as glucose and glycogen stores in the liver, as early as 49 days post-infection (Wang *et al.,* 2004). Under such nutrient-poor conditions in the host, one of the main results of increased consumption of energy is an increase in AMP and ADP, consistent with the observation in the current study (Carling *et al.,* 2012, Hardie *et al.,* 2012). These molecules act as sensors for energy homeostasis that help activate alternative pathways such as glycogenolysis, gluconeogenesis, and fatty acid synthesis in attempts to replenish energy stores. Also consistent with the observed increases

in AMP in such nutrient-poor conditions, is the enhanced energy demand for increased protein synthesis, especially for tRNA activation and guanosine triphosphate regeneration, which results in direct dephosphorylation of even more ATP to AMP by the host system (Pontes *et al.,* 2015). Parallel to the increased glycolysis, the increased levels of G6P and 3-PG enhances the oxidative phase of the PPP (oxidising even more glucose to produce energy) to generate nicotinamide adenine dinucleotide phosphate (NADPH) for host anabolic reactions, including the biosynthesis of nucleic acids and fatty acids (Eggleston and Krebs 1974). Although this remains a pathway-based interpretation, the observed alteration in energy metabolism pathways including glycolysis and the PPP, are in line with current understanding that such pathways of glucose utilisation are predominantly stimulated during schistosome infection (Ahmed and Gad 1995, Wang *et al.,* 2004, Wu *et al.,* 2010b). Moreover, evidence from experimental studies show that the liver injury caused by schistosome infection (Wu *et al.,* 2010b) is marked by stimulated host glycolysis, manifested by reduced plasma glucose as well as glucose and glycogen stores in the liver (Wang *et al.,* 2004). Taken together, the results suggest an interplay between the host and schistosome worms, consistent with the parasite-related morbidity observed in young children, including malnutrition, poor growth, and poor physical and cognitive performance (Freer *et al.,* 2018).

Schistosome worms have essential phosphatases and phosphohydrolases such as SmAP, a schistosome tegumental alkaline phosphatase that cleave exogenous phosphates to generate various reaction products (Cesari *et al.,* 1981, Bhardwaj and Skelly 2011). In addition, schistosome worms lack *de novo* synthesis of purines and resort to salvaging molecules from the host using these tegumental phosphatases (Levy and Read 1975a), through dephosphorylation and subsequent uptake of reaction products (Levy and Read 1975b). Thus, the observed increases in AMP, ADP, and G6P are important for parasite purine and energy uptake, via phosphate cleavage using tegumental enzymes. The resulting exogenous molecules such as adenosine have anti-inflammatory properties known to dampen host immunity (Hasko

and Cronstein 2004, Bours *et al.,* 2006, Gessi *et al.,* 2007). For instance, adenosine inhibits the production of pro-inflammatory cytokines and inflammatory mediators (Hasko *et al.,* 2000), inhibits lymphocyte adhesion, and attenuates the proliferative and cytotoxic responses of activated T-cells (Hoskin *et al.,* 2002, Zhang *et al.,* 2004). This would benefit schistosome parasites by creating a less inflammatory and immunologically friendly environment, key to survival of the parasite in the host. Data suggests that such essential functions of parasite phosphatases occur *in vivo* but less *in vitro*, consistent with the hypothesis of the benefits of such molecules for schistosome survival in the host (Bhardwaj and Skelly 2011). Another direct benefit of the enhanced PPP in generating molecules for fatty acid synthesis in the host is that, schistosomes rely on scavenging lipid precursors from the host to generate phospholipids, due their inability to synthesise fatty acids *de novo* (Meyer *et al.,* 1970, Brouwers *et al.,* 1997). Schistosome lipids have also been demonstrated to stimulate immunomodulation of the host to enhance parasite survival (van der Kleij *et al.,* 2002, Giera *et al.,* 2018).

The correlation between metabolic alterations and increasing infection intensity is consistent with experimental studies of metabolic changes being linked to disease progression in schistosome infection (Li *et al.,* 2009, Wu *et al.,* 2010b). In addition, curative treatment with PZQ in schistosome-positive children showed a decrease in levels of the altered metabolites, 3 months post-treatment. Consistent with normalisation of affected pathway enzymes upon treatment of schistosome infection in mice models (Ahmed and Gad 1995), this strengthens the idea that the observed changes are in response to or related to schistosome infection (Wu *et al.,* 2010b). This observation is also consistent with the fact that curative treatment results in the reversal of early schistosome morbidity/pathology (Wami *et al.,* 2016), and with suggestions that catch-up growth and development is possible in children, following curative PZQ treatment (Gurarie *et al.,* 2011). It is thus plausible that the observed effect of curative treatment would have been more marked, had the children been surveyed for longer than 3 months post-treatment. This is because it may take longer than 3 months for metabolite levels to return to pre-infection levels.

The strength of the current study is that the study design allowed for analysis of matching pre- and post-infection samples, to gain understanding into the early metabolic responses leading to morbidity from the first schistosome infection in young children who had never been infected. However, it was limited to the fact that following a natural time-course of first schistosome infections meant there was no control over the number of infected or uninfected children included in the study post-baseline, hence smaller sample sizes especially for schistosome positive individuals. The urine filtration technique used for diagnosis of *S. haematobium* infection in the current study is less sensitive to detecting very low intensity, pre-patent or single sex infections. However, the current study allows comparison with other studies while parasitological egg count methods remain the predominant schistosome diagnostic in PSAC. Long-term studies relating measurable clinical manifestations of schistosome infection in children to such metabolic alterations, would give a stronger indication of the clinical implications of the schistosome-induced metabolic disturbances.

5.6 Conclusions

I showed that in a cohort of Zimbabwean PSAC (≤5 years old), the first infection with *S. haematobium* is associated with significant alterations in host energy and purine metabolism, early in infection. These changes correlated with infection intensity and resolved 3 months post-curative antihelminthic treatment. The findings suggest an interplay between the host and schistosome worms, consistent with parasite survival, disease progression, and parasite-related morbidity including malnutrition, poor growth, and poor physical and cognitive performance in schistosome-infected children. The findings in this chapter will inform development of appropriate interventions in human helminth infections, such as nutraceuticals in child feeding programs, targeted at reducing morbidity associated with the disease. Further mechanistic studies will contribute to more understanding of the role of metabolic alterations in the aetiology of schistosome-related pathology in children.

Chapter 6 Differences in the gut microbiome and resistome in *Schistosoma haematobium* infected vs. uninfected preschool-aged children

Part of this work has been published (Osakunor *et al.,* 2020) and a copy of the publication is included in **Appendix E**.

6.1 Introduction

There is increasing evidence that humans rely on the gut microbiota (a diverse ecosystem of microbes) (Lukes *et al.,* 2015) for essential functions including extracting essential nutrients from food, as a first line of protection from enteric pathogens, and as a mechanism for shaping the immune system (Human Microbiome Project Consortium 2012). These essential functions are however not facilitated by the same microbiota in all people (Shafquat *et al.,* 2014). Large scale projects that have characterised the microbiome (the assembly of genomes of the microbiota) have shown that considerable heterogeneity exist among different human populations (Turnbaugh *et al.,* 2007, Human Microbiome Project Consortium 2012).

Studies have shown that the composition of the human gut microbiome is influenced by age (Yatsunenko *et al.,* 2012, Rodriguez *et al.,* 2015), diet and geography (De Filippo *et al.,* 2010, Fan *et al.,* 2014, Senghor *et al.,* 2018), host genotype (Goodrich *et al.,* 2014), exposure to maternal microbiota (Dominguez-Bello *et al.,* 2010), as well as environmental factors (Martinez *et al.,* 2015) including the role of protozoal and helminth parasites (Mishra *et al.,* 2014). However, populations in Africa have been underrepresented in microbiome studies, with a major focus on Western populations (Brewster *et al.,* 2019). Limited number of studies have included African populations, but with a focus on elucidating the impact of geographical and lifestyle differences on the gut microbiome of African and Western populations (De Filippo *et al.,* 2010, Yatsunenko *et al.,* 2012, Rampelli *et al.,* 2015). Studies that have focused on African populations have included older individuals (i.e. 20–40 years old) whose

microbiome structure (abundance and diversity) is already established (Schnorr *et al.,* 2014), not allowing the factors inherent to African childhood to be fully disentangled.

In Africa, children are exposed to several acute and chronic parasitic infections that can impact significantly on their growth and development (Bustinduy *et al.,* 2013). In particular, schistosome worms can be contracted by children as young as 6 months of age (Mafiana *et al.,* 2003, Bosompem *et al.,* 2004). Consequences of schistosome infection at this young age can include malnutrition, poor growth and cognitive development, as well as susceptibility to, and altered prognosis of coinfections (van der Werf *et al.,* 2003, Freer *et al.,* 2018). From birth, the gut microbial population continues to evolve until about age 3–5 years (Yatsunenko *et al.,* 2012, Rodriguez *et al.,* 2015). Therefore, infections that young children are exposed to can influence the establishment of the microbiome, and has implications on overall health and disease (Laforest-Lapointe and Arrieta 2017). Microbiome research focused on preschool-aged children (PSAC), i.e. 5 years old and below, will provide further insight for influencing health through the microbiome in infants and young children (Robertson *et al.,* 2019).

Evidence shows that schistosome infections acquired at the preschool age can persist into the second decade of life where they modulate the immune system to promote parasite survival, and cause long-term disease; reviewed by McManus *et al.,* (2018). There are suggestions that the immunomodulatory effects of schistosome infection can extend to the gut microbiota through indirect systemic interactions (Mishra *et al.,* 2014). Correlations between the gut microbiome and systemic diseases such as rheumatoid arthritis suggest the importance of such systemic interactions (Scher *et al.,* 2013). Recently, fluctuations in the composition of the gut microbiota of mice infected with *S. mansoni*, before and after intestinal damage from egg transmission was shown (Jenkins *et al.,* 2018). Furthermore, depletion of the gut bacteria was associated with reduced *S. mansoni* egg excretion, gut pathology and inflammation in mice (Holzscheiter *et al.,* 2014). This is consistent with the role of the mammalian gut microbiota in the pathogenesis of schistosomiasis. However, unlike *S. mansoni* (intestinal form) that

inhabits blood vessels close to the same environment as the gut microbiota, *S. haematobium* predominantly resides in the venous plexus of the bladder (although it has occasionally been found in the mesenteric circulation (Cheever *et al.,* 1977)), and thus presents a need for further studies into the indirect systemic impacts of the infection on the gut microbiota.

Much of our understanding of the helminth interactions with the gut microbiota in humans has been facilitated by experimental studies (as discussed in **Chapter 1**), and phenotypic and mechanistic studies from natural human infections are limited. A recent study conducted in children 13 years old and below reported differences in gut microbiota abundance between *S. haematobium* infected versus uninfected children, suggesting the presence of different microbial communities during schistosome infection (Kay *et al.,* 2015). This was supported by Schneeberger *et al.,* (2018) in an *S. mansoni* study, suggesting that genetic and environmental factors may play an additional role. More recently, a study conducted among older children (11–15 years) in Nigeria suggested that microbiome disruptions may be a further consequence of urogenital schistosomiasis (Ajibola et al., 2019). However, substantial knowledge gaps on the interaction between the gut microbiota and schistosome infection in PSAC still exist (Mutapi 2015b). Further studies into the associations between human schistosome infection and the gut microbiota in PSAC are important to gain insight into variations in infection/disease burden and patterns. It will also provide insight into opportunities to design treatment regimens to improve overall child health and development. The biggest challenges are in the ability to demonstrate causation and elucidate mechanistic pathways for any existing interactions.

6.1.1 The resistome in microbiome analysis

Antimicrobial resistance (AMR) to bacteria is one of the largest threats to human health, and occurs when bacteria adapt to exposure and treatment with antibiotics (Wise and Soulsby 2002). The microbiome is a reservoir for AMR genes (collectively known as the resistome)

(Qin *et al.,* 2010, Hu *et al.,* 2013), and provides an ideal environment for AMR gene exchange among the "resident" and transitory bacterial populations (Jernberg *et al.,* 2010), contributing to AMR. Thus, it is plausible that any alterations in the gut microbiota could have an impact on AMR gene abundance and diversity. In examining the impact of interactions between helminth infections, including schistosomiasis, on the composition of the gut microbiome, it is important to examine other interactions relevant to the health of the host, including impacts on the resistome. One such example is the ability of *Salmonella* to persist in the body by attaching to intestinal schistosomes and evading repeated antibiotic treatments, as demonstrated by experimental studies (LoVerde *et al.,* 1980, Barnhill *et al.,* 2011). This phenomenon can lead to an increase in the *Salmonella* population, and eventually, potential antibiotic resistance from persistent antibiotic treatment (Barnhill *et al.,* 2011).

While AMR remains one of the largest threats to human health (Qin *et al.,* 2010, Doron and Davidson 2011, De Waele *et al.,* 2018), the majority of AMR gene studies are conducted in industrialised settings (Hu *et al.,* 2013, Wellington *et al.,* 2013, Woolhouse and Ward 2013, Blair *et al.,* 2015, Holmes *et al.,* 2016), which contrast with low- and middle-income countries in terms of access to safe water and sanitation, as well as access to antibiotics, with or without prescriptions (Okeke *et al.,* 2005). The situation in developing countries is further compounded by complex socio-economic and behavioural factors (Okeke *et al.,* 1999, Ayukekbong *et al.,* 2017).

6.2 Study aims

The aim of this chapter was to characterise in a cohort of PSAC (1–5 years old), the abundance and diversity of the gut microbiota (including the fungi repertoire) and AMR genes, and to determine the association between *S. haematobium* infection and the gut microbiome and resistome. In addition, I determined how this relationship is influenced by host-related factors including socio-demography, growth and nutritional indices, as well as clinical history. The findings in this chapter add to understanding into schistosome infection and its association

with the gut microbiome. The chapter further adds to the repository of information on gut microbiome and AMR studies in PSAC in sub-Saharan Africa.

6.3 Hypotheses

(i) *Schistosoma haematobium* infection is associated with alterations in abundance and diversity of the gut microbiota in PSAC.

(ii) The schistosome-associated alterations in gut microbiota abundance and diversity are commensurate with schistosome infection intensity.

(iii) The schistosome-associated alterations in the gut microbiota are associated with AMR gene abundance and diversity.

6.4 Methods

6.4.1 Study design, population and site

This was a cross-sectional study that included stool samples from a subset of 116 PSAC (age range 1–5 years), meeting the inclusion criteria as part of the baseline survey for the larger study described in **Chapter 2**. Children were selected from Mupfure and Chihuri villages. Metadata on socio-demography (including age, sex, village), growth and nutrition (including weight, height and feeding habits), and clinical history (including history of schistosome treatment and antibiotic use) gathered from questionnaires administered at the time of recruitment were used (details in **Chapter 2**). Clinical records were checked to obtain history of antibiotic use, within the 6 months preceding acquisition of stool samples. Children who tested positive for *S. mansoni* and soil-transmitted helminths (STH) as part of the baseline survey were excluded from the subset of 116 children selected. Thus, children were selected based on: (i) consent from parents/guardians, (ii) availability of socio-demographic data, (iii) availability of parasitology samples (urine and stool samples), (iv) availability of test results and clinical history, and (v) no current episodes of diarrhoea (assessed by questionnaire and visual stool examination).

There are limited schistosome–microbiome studies focusing solely on PSAC to inform sample size calculations. However, the recent studies conducted in older children (Kay *et al.,* 2015, Schneeberger *et al.,* 2018, Ajibola *et al.,* 2019) show that sample sizes ranging from 34–139 is sufficient to detect significant differences in the microbiome of schistosome-infected versus uninfected children.

6.4.2 Sample collection and processing

Urine and stool samples were collected for parasitological diagnosis of *S. haematobium* and to exclude intestinal helminths respectively (*S. mansoni* and STH) as described in **Chapter 2**. All children who were positive for schistosome infection were treated with a single dose of praziquantel (PZQ) at the standard 40 mg/kg body weight (details in **Chapter 2**). DNA was extracted from aliquots of stool samples and processed for library preparation and metagenomic sequencing at the Beijing Genomics Institute (BGI, Shenzhen, China), as described in **Chapter 2**.

6.4.3 Quality control and trimming

Raw FASTQ format (a text-based format for biological sequence outputs) sequences from each sample were quality assessed using FASTQC v0.10.0 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The output of the FASTQC analysis showed that the raw FASTQ sequences were of good quality, and the number of read pairs generated per sample ranged from 9,263,538 to 21,350,613. Subsequently, reads were trimmed, to include removing adaptors, using BBduk2 (BBMap—Bushnell B.— https://sourceforge.net/projects/bbmap/) with an output quality Phred threshold score of ≥20 and a minimum read length of 50 bp. K-mer length for finding contaminants was k=19 to include shorter k-mers at read pairs down to k=11, and reads were trimmed at the right end.

6.4.4 Mapping of sequence reads

Mapping of sequence reads was done using the method of k-mer alignment (KMA), as described in **Chapter 2**. To access the microbiome sequence component present in the samples, read pairs and singletons were aligned to a custom reference genomic database (last updated 04.04.2019). For both read pairs and singletons, mapped reads were counted as one copy. Unless otherwise specified below, databases were primarily downloaded via the National Center for Biotechnology Information (NCBI) GenBank clade specific assembly_summary.txt files (ftp://ftp.ncbi.nlm.nih.gov/genomes/genbank). The custom database consisted of the following: bacteria (closed genomes; downloaded 05.02.2019), archaea (downloaded 13.02.2019), MetaHitAssembly (PRJEB674–PRJEB1046; downloaded 01.07.2014), HumanMicrobiome (genome assemblies; downloaded 02.07.2014), bacteria_draft (downloaded 05.02.2019), plasmid (downloaded 05.02.2019), virus (https://bitbucket.org/genomicepidemiology/kvit_db; downloaded 05.02.2019; https://genome.jgi.doe.gov/portal/pages/dynamicOrganismDownload.jsf?organism=IMG_VR; downloaded 28.01.2019), fungi (downloaded 13.02.2019), protozoa (downloaded 13.02.2019), and parasites (downloaded 04.04.2019). Sequences selected for the bacteria and bacteria_draft databases from the assembly_summary.txt file, were annotated with the tags version_status= 'latest' and genome_rep= 'Full'. Additional entries, assembly_level= 'Complete genome' or 'Chromosome' in the bacteria database and refseq_category= 'representative genome' in the bacteria_draft database were also required. The plasmid database was constructed as a subset of the bacteria and bacteria_draft sequences; keyword in the FASTA entry header line, 'plasmid'. The total read count for each microbial community of interest in a sample was calculated as the sum of read counts from each of the databases of interest; bacteria (bacteria, bacteria_draft, MetaHitAssembly, and HumanMicrobiome), fungi, protozoa, and parasites.

The primary (most similar) alignment obtained for mapped sequences was used to assign a taxonomy, based on the taxonID obtained. TaxonID's and associated taxonomy classifications

were obtained from downloaded reference microbial genomes from NCBI (ftp://ftp.ncbi.nih.gov/pub/taxonomy/taxdump.tar.gz) and assignment at all taxonomic levels was done. Sequences that had no similarities detected in the nucleotide (nt) database to assign a taxonomic classification were deemed to be unknown sequences, and hence termed "unknown". The classification of "unknown" in this case is time-sensitive but was appropriate and correct at the time of analysis.

To identify AMR genes present in the samples, read pairs were aligned to AMR genes (3081 genes) present in the ResFinder database (https://bitbucket.org/genomicepidemiology/resfinder_db; downloaded 13.02.2019). Parameters were set for a query gene to cover at least 2/5 the length of the reference gene in order to be selected (Zankari *et al.,* 2012). Alignments were filtered to retain those exhibiting a selected threshold of identity of 90% (i.e. >90% nucleotide identity between the query and reference gene over at least 90% of the length of reference gene). Zankari *et al.,* (2017) showed that the minimum threshold percentage length of the resistance gene to be covered was 60%, and with ResFinder, a possibility to search for genes with specified similarity from 80%–100% identity (Zankari *et al.,* 2012). This is to ensure that unlike with the default 100% identity, AMR genes identified spread over two contigs and/or that sequences located on the ends of the contig were not missed. For instance, resistance to some antimicrobial agents, in particular fluoroquinolones, can be caused by point mutations (Single Nucleotide Polymorphisms (SNPs)), and Zankari *et al.,* (2017) have used PointFinder to detect point mutations known to confer antibiotic resistance. The threshold used in the current study (i.e. 90% identity and 90% coverage) thus ensured that while reducing noise associated with lower thresholds (e.g. mapping to fragments of genes), there was not only a higher chance of finding the appropriate genes but also finding them accurately.

6.4.5 Data handling and processing

To accurately explain variations in the data using available participant metadata, three samples were excluded from the final data analysis using predefined criteria. Samples were included in data analysis provided they had at least one metadata variable from growth and nutritional indices, schistosome infection status, previous schistosome treatment, and antibiotic use data were used for all downstream analyses (i.e. n=113). To account for probable sample-wise sequencing depth differences, as well as a size-dependent probability of observing a reference, mapping counts from the custom genomic database and from the ResFinder database were normalised to the total genome sizes for the genomic database, and to the individual gene lengths for the ResFinder database.

The total observed mapping counts are relative, and may account for confounding effects on downstream analyses (McMurdie and Holmes 2014). This may be due to limitations of an arbitrary total imposed by different sequencing platforms, technical variations in sequencing libraries amounts, or even random variation (Gloor *et al.,* 2017). Thus, to obtain information about the abundances of features in the dataset relative to each other, datasets were treated as compositional (Gloor *et al.,* 2017). Data were transformed using the log-ratio approach as introduced by Aitchison, to make the data symmetric, linear and in a log-ratio coordinate space (Aitchison 1986). However compositional methods such as this do not account for the presence of zeros associated with abundance datasets. To address this, a small pseudo count of half the smallest non-zero abundance per feature was added to each respective feature for all the normalised abundance matrices, prior to transformations (Calle 2019). Microbiota abundance data tables with counts, *x*, and *k* number of populations (taxa members), were centred log-ratio (clr) transformed, defined as (Calle 2019):

$$clr(x_1, \cdots, x_k) = \left(\log\left(\frac{x_1}{g_{(x)}}\right), \cdots, \left(\log\left(\frac{x_k}{g(x)}\right)\right)\right) \quad (6.1)$$

Where, $g(x) = (\prod x_i)^{1/k}$ is the geometric mean of the particular composition.

AMR gene abundances were additive log-ratio (alr) transformed, taking the bacterial component of the microbiome (x_k) as the reference, defined as (Calle 2019):

$$alr(x_1, \cdots, x_k) = \left(\log\left(\frac{x_1}{x_k}\right), \cdots, \left(\log\left(\frac{x_{k-1}}{x_k}\right)\right)\right) \quad (6.2)$$

Unless otherwise stated, clr and alr matrices were used for all downstream analyses.

6.4.6 Visualization

Data visualization was done using R (www.bioconductor.org; www.r-project.org) (R Development Core Team 2011). Bar plots from normalised, zero-corrected abundance matrices, were used to give an overview of the microbiota and AMR gene abundances across all samples. For cluster dendrograms, the Aitchison distance (Euclidean distance) was calculated using clr-transformed abundance data, and samples clustered based on distances (Complete-linkage-clustering). To explore underlying variabilities in the microbiota and AMR genes across the data set, clr-transformed abundance data for each matrix, centred on the geometric sample mean and scaled by the total variance was ordinated using Principal Component Analysis (PCA) (Calle 2019), based on eigenvectors and eigenvalues (Arfken 1985). Box plots were used to highlight differences in microbiota abundance between two groups, and scatter plots to show the relationship between schistosome infection intensity and microbiota abundance.

6.4.7 Statistical analyses

Data were analysed using various Bioconductor packages in R software. Details of the statistical methods used here are described in **Chapter 2**.

To test whether sample-related metadata were significant predictors of within-group dispersion of the microbiota and AMR genes, the Euclidean distances were calculated, using the R/Bioconductor package vegan (Oksanen *et al.,* 2016). The effect of such metadata on sample dissimilarities were determined using permutational multivariate analysis of variance

(PERMANOVA; adonis2 function in the vegan package) using $p < 0.05$ as the significance threshold. A Benjamini–Hochberg False Discovery Rate (FDR) correction was applied to counteract multiple testing (Benjamini and Hochberg 1995).

To investigate further how specific taxa composition vary across the statistically significant metadata (from PERMOANOVA), while controlling for other variables of interest, analysis of composition of microbiomes (ANCOM) was used (Mandal *et al.,* 2015). Analysis was based on log-ratio transformation of raw count data (clr), where the normalizing reference value is the abundance of all remaining taxa, taken one at a time. The FDR was set at 0.05 (Benjamini and Hochberg 1995), and a taxa member was considered at a W-statistic cut off, 0.80. The ANCOM test for the association of *S. haematobium* infection was controlled for age, sex and village.

As ANCOM only provides a list of taxa that vary in composition, the magnitude and direction of associations of taxa that vary in composition across independent variables was further determined. Box plots stratified by specific independent variables, using the clr-transformed abundance data of significant taxa previously identified by ANCOM were used to highlight differences in groups. To determine how these taxa varied with schistosome infection intensity, clr-transformed abundance data was regressed on the log-transformed infection intensity (log10 [egg count+1]).

6.4.8 Data availability

Raw sequence data files from all 116 samples and associated metadata used in the current study are deposited in the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information (NCBI) database under the BioProject accession number PRJNA521455. All other data files including QC, sequence and Taxon ID mapping information (downloadable from: https://static-content.springer.com/esm/art%3A10.1038%2Fs42003-020-0859-7/MediaObjects/42003_2020_859_MOESM3_ESM.xlsx), and base

data from which all statistical analyses were done (downloadable from: https://static-content.springer.com/esm/art%3A10.1038%2Fs42003-020-0859-7/MediaObjects/42003_2020_859_MOESM4_ESM.xlsx) are published in Osakunor *et al.,* (2020).

6.4.9 Code availability

The updated R codes used for ANCOM are available on, https://github.com/zellerlab/crc_meta/blob/master/src/ANCOM_updated.R or from the author's webpage at https://sites.google.com/site/siddharthamandal1985/research.

6.5 Results

6.5.1 Population characteristics

Of the 113 participants included in the current study, the mean age was 3.7 ± 1.1 years, of which 56 (49.6%) were female and 57 (50.4%) were male. Sixty-eight (60.2%) and 45 (39.8%) children were from Chihuri and Mupfure villages respectively. Antimicrobial use data showed that 58 (51.3%) participants had received antibiotics [amoxicillin (31), co-trimoxazole (27), both (9)], while 18 (15.9%) had not; no information was obtained for the remaining 37 (32.7%) participants. Previous history of PZQ treatment was reported among 29/105 (27.6%) children. *Schistosoma haematobium* infection prevalence was 15.9% (18/113), with mean infection intensity of 1.79 eggs/10 ml urine (SEM= 0.76; range= 0–74).

Data on the history of feeding habits and nutritional status showed that the majority (85.8%; 97/113) were breastfed, with duration ranging from 2 to 48 months (median= 18 months IQR: 17–20). Children were introduced to solid foods from between 1 to 24 months after birth. Diet comprised mainly of traditional maize flour porridges (97%; 96/103), the commercial Cerelac® porridge (1.9%; 2/103), and potatoes (1%; 1/103). Anthropometric measures, adjusted for age, were used to assess nutritional status as described in **Chapter 2**. Based on the weight-for-height Z-scores (WHZ), 3.7% (4/107) of individuals were malnourished, and

14.7% (16/109) were stunted based on the height-for-age Z-scores (HAZ) (MOH Malawi 2016).

6.5.2 Taxonomic composition of the microbiome

The number of classified read pairs per sample ranged from 3,994,704 to 13,164,482. An average 45.1% of read pairs were mapped to specific reference sequences in the genomic database; this is similar to other studies with the proportion of unmapped reads ranging from 42%–68% (Afshinnekoo *et al.,* 2015, Nordahl Petersen *et al.,* 2015, Hendriksen *et al.,* 2019). At any taxonomic level, a taxonomic classification could not be assigned to at least 33% of the mapped read pairs and were thus classified as "unknown".

In the 113 stool samples, 845 bacteria genera (from 20 unique phyla), and 228 fungi genera (from 6 unique phyla) were detected. As shown in **Figure 6.1**, the most abundant bacteria phyla in decreasing order were *Bacteroidetes* (genera: *Prevotella*, *Bacteroides*, *Alistipes*), *Firmicutes* (genera: *Eubacterium*, *Faecalibacterium*, *Clostridium*, *Roseburia*), and *Proteobacteria* (genus: *Succinatimonas*). The most abundant fungi phyla were *Ascomycota* (genera: *Protomyces*, *Aspergillus*, *Taphrina*, *Saccharomyces*, *Candida*, *Nakaseomyces*), *Microsporidia* (genus: *Enterocytozoon*), and *Zoopagomycota* (genus: *Entomophthora*) [see **Figure 6.2**]. These phyla dominated the microbiome and were present in all samples.

Figure 6.1: Overview of the bacterial microbiota abundance and diversity
From read mapping to the genomic database, abundance was calculated for each microbial taxon across all samples. Stacked bar charts show the most abundant bacteria a) phyla and b) genera per sample, proportional to the total microbiota within each sample (n=113 biologically independent samples). Charts were generated using normalised, zero-corrected abundance matrices. "Unknown" represents abundance data for which a taxonomic classification could not be assigned. "-Others" represents abundance data for all other taxa in the abundance data set.

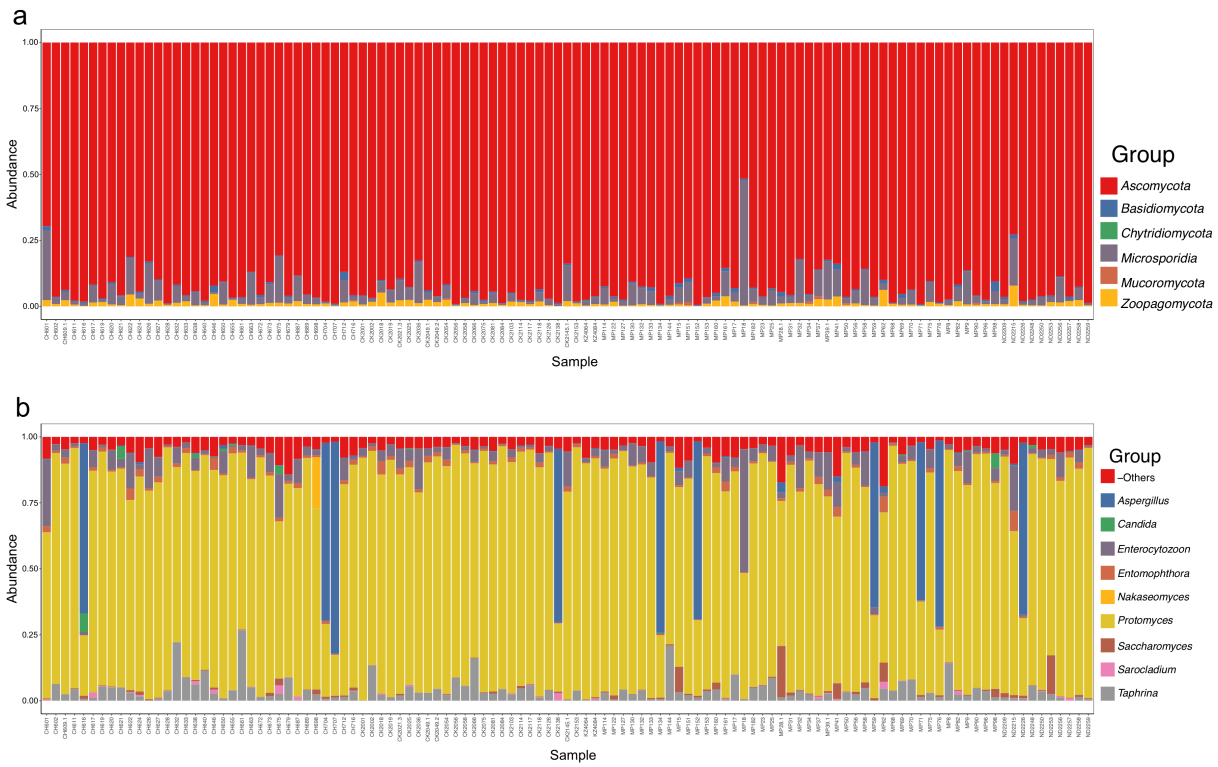

Figure 6.2: Overview of the fungal microbiota abundance and diversity
From read mapping to the genomic database, abundance was calculated for each microbial taxon across all samples. Stacked bar charts show the most abundant fungi a) phyla and b) genera per sample, proportional to the total microbiota within each sample (n=113 biologically independent samples). Charts were generated using normalised, zero-corrected abundance matrices. "-Others" represents abundance data for all other taxa in the abundance data set.

6.5.3 Variation in the microbiome and association with participant metadata

PCA was used to initially examine variability and patterns in the data set across the first two principal components. At the phylum level, PCA explained 62% and 42.0% of the total variation in fungi and bacteria respectively. At the genus level however, PCA explained 34% and 16% of the total variation in fungi and bacteria respectively. The model showed homogeneity in components with no distinct clustering according to metadata and may reflect a high diversity in the cohort. PCA plots and cluster dendrograms for bacteria and fungi content per sample is shown in **Supplementary Figures 1–3**, **Appendix C**.

PERMANOVA analysis showed a significant effect of age (FDR= 0.024), village (FDR= 0.039), schistosome infection status (FDR= 0.039), and schistosome infection intensity (FDR= 0.012) on bacteria genera, across samples. There was also a significant effect of schistosome infection status (FDR= 0.006) and schistosome infection intensity (FDR= 0.006) on fungi genera, across samples. For both bacteria and fungi genera, no such effects were found for sex, nutritional and growth variables, feeding, previous PZQ treatment, and antibiotic use (FDR >0.05). Summary output from the analysis is shown in **Table 6.1**.

Table 6.1: Model summaries of participant metadata and association with the microbiome

		Bacteria				Fungi			
Variable	**n**	**p-value**	**SS-E**	**SS Total**	**FDR**	**p-value**	**SS-E**	**SS Total**	**FDR**
Age (years)	113	0.004	1344.6	82733.4	**0.024**	0.082	128.6	9489.2	0.197
Sex	113	0.172	878.1	83200.0	0.258	0.439	82.8	9534.9	0.671
Village	113	0.012	1254.0	82824.0	**0.039**	0.060	140.4	9477.4	0.180
***S. h.* infection status (pos/neg)**	113	0.013	1185.1	82892.9	**0.039**	0.001	339.0	9278.7	**0.006**
***S. h.* infection intensity**	113	0.001	1514.5	82563.6	**0.012**	0.001	670.1	8947.7	**0.006**
Malnourished, yes/no (WHZ)	107	0.866	589.1	78498.2	0.913	0.830	59.5	9145.6	0.830
Stunted, yes/no (HAZ)	109	0.407	754.6	79751.0	0.542	0.611	71.5	9227.2	0.671
Months breastfed	90	0.082	954.9	64235.8	0.140	0.470	75.2	6985.0	0.671
Months solid food introduced	102	0.913	573.1	75583.0	0.913	0.615	73.5	8792.0	0.671
Previous PZQ treatment	105	0.071	991.9	77387.4	0.140	0.233	101.6	8934.5	0.466
Amoxicillin (yes/no)	76	0.771	646.6	55603.4	0.913	0.531	78.4	6567.3	0.671
Co-trimoxazole (yes/no)	76	0.030	1083.6	55166.4	0.072	0.048	158.3	6487.4	0.180

Table represents PERMANOVA output for bacteria and fungi genera. Classification of nutritional status was based on a cut off <-2 Z–scores (MOH Malawi 2016). Schistosome infection intensity was log transformed (log10 [egg count+1]). *S. h*., *S. haematobium*; WHA, weight–for height Z-scores; HAZ, height-for-age Z-scores; pos/neg, positive /negative; p-value, unadjusted p-value; FDR, adjusted p-value (FDR-corrected); SS-E, explained sum of squares; SS Total, total sum of squares.

6.5.4 Microbiome analysis by child age and village

From the PERMANOVA results, further analysis via ANCOM showed that the abundance of six specific bacteria genera were associated with age and village. *Intestinibacter* (W= 315), *Mycoplasm* (W= 298), and *Turicibacter* (W= 293) showed significant variation across age, and *Senegalimassilia* (W= 312), *Mordavella* (W= 296), and *Azospirillum* (W= 281), showed variation with village. The magnitude of the differences in abundance between groups are shown in **Figure 6.3**. Abundance of the identified genera did not increase or decrease as children grew per se, but rather, abundance was variable across all ages 1–5 years. Abundance of all the identified genera that varied with village was higher in Mupfure than in Chihuri.

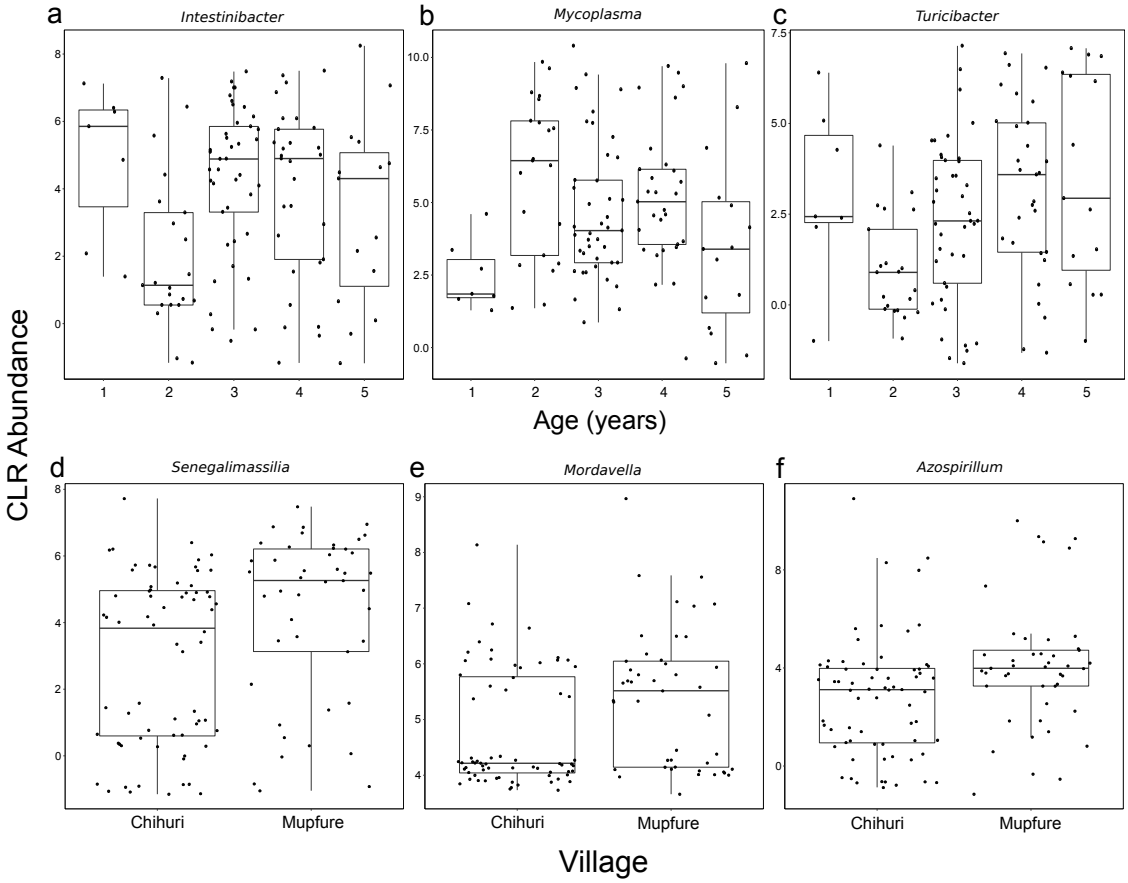

Figure 6.3: Bacteria genera whose abundance vary significantly across host age and village
Figure a–c) Box plots showing the abundance of specific bacteria genera, grouped by age category in years. Figures d–f) Box plots showing the mean abundance of specific bacteria genera, grouped by village. Specific groups are represented on the x-axis and the abundances (clr-transformed) shown on the y-axis. The horizontal box lines represent the first quartile, the median and the third quartile. Whiskers denote the range of points within the first quartile − 1.5× the interquartile range and the third quartile + 1.5× the interquartile range.

6.5.5 Microbiome analysis by schistosome infection status and intensity

ANCOM was used to determine specific bacteria and fungi genera abundance associated with *S. haematobium* infection, while controlling for age, sex and village. This was followed by evaluation for association with schistosome infection intensity. In total, eight genera were identified, five of which were bacteria (*Pseudomonas;* W= 347, *Azospirillum*; W= 346, *Stenotrophomonas*; W= 292, *Derxia*; W= 288, and *Thalassospira*; W= 292) and three were fungi (*Aspergillus*; W= 75, *Tricholoma*; W= 73, and *Periglandula*; W= 70). The magnitude of the differences in abundance between *S. haematobium*-infected and uninfected children are shown in **Figure 6.4** and **Figure 6.5.** In schistosome-positive children, the abundance of all but *Azospirillum* was higher (**Figure 6.4 a–e**). This observation was consistent with infection intensity as shown in **Figure 6.4 f–j** [*Pseudomonas* (r= 0.3; p=0.001), *Azospirillum* (r= -0.4; p <0.001), *Stenotrophomonas* (r=0.4; p <0.001), *Derxia* (r= 0.6; p <0.001), and *Thalassospira* (r= 0.6; p <0.001)]. Likewise, the abundance of *Aspergillus*, *Tricholoma*, and *Periglandula* was higher in schistosome-positive children (**Figure 6.5 a–c**) and was consistent with infection intensity as shown in **Figure 6.5 d–f** [*Aspergillus* (r= 0.5; p <0.001), *Tricholoma* (r= 0.5; p <0.001), and *Periglandula* (r= 0.4; p <0.001)].

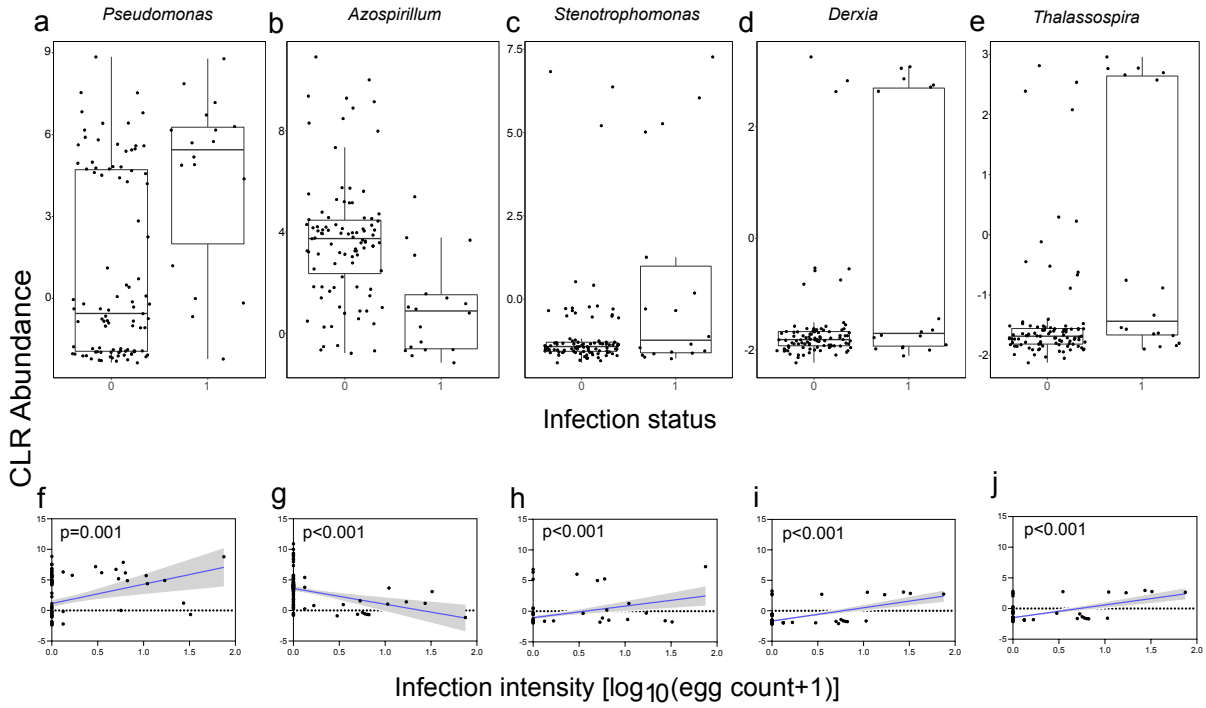

Figure 6.4: Bacteria genera whose abundance vary significantly with schistosome infection
Figure a–e) Box plots showing the abundance of specific bacteria genera, grouped by *S. haematobium* infection status. The horizontal box lines represent the first quartile, the median and the third quartile. Whiskers denote the range of points within the first quartile − 1.5× the interquartile range and the third quartile + 1.5× the interquartile range. Figures f–j) Scatter plots showing linear regression analysis of *S. haematobium* infection intensity and bacteria genera abundance. Figures f–j are plots matching the same genera shown in figures a–e. The clr-transformed abundance data was used for all plots. Infection status was coded as 0 and 1 for negative (n=95) and positive (n=18) respectively. *S. haematobium* infection intensity was log transformed [log10 (egg count+1)]. Shaded areas indicate the 95% confidence intervals.

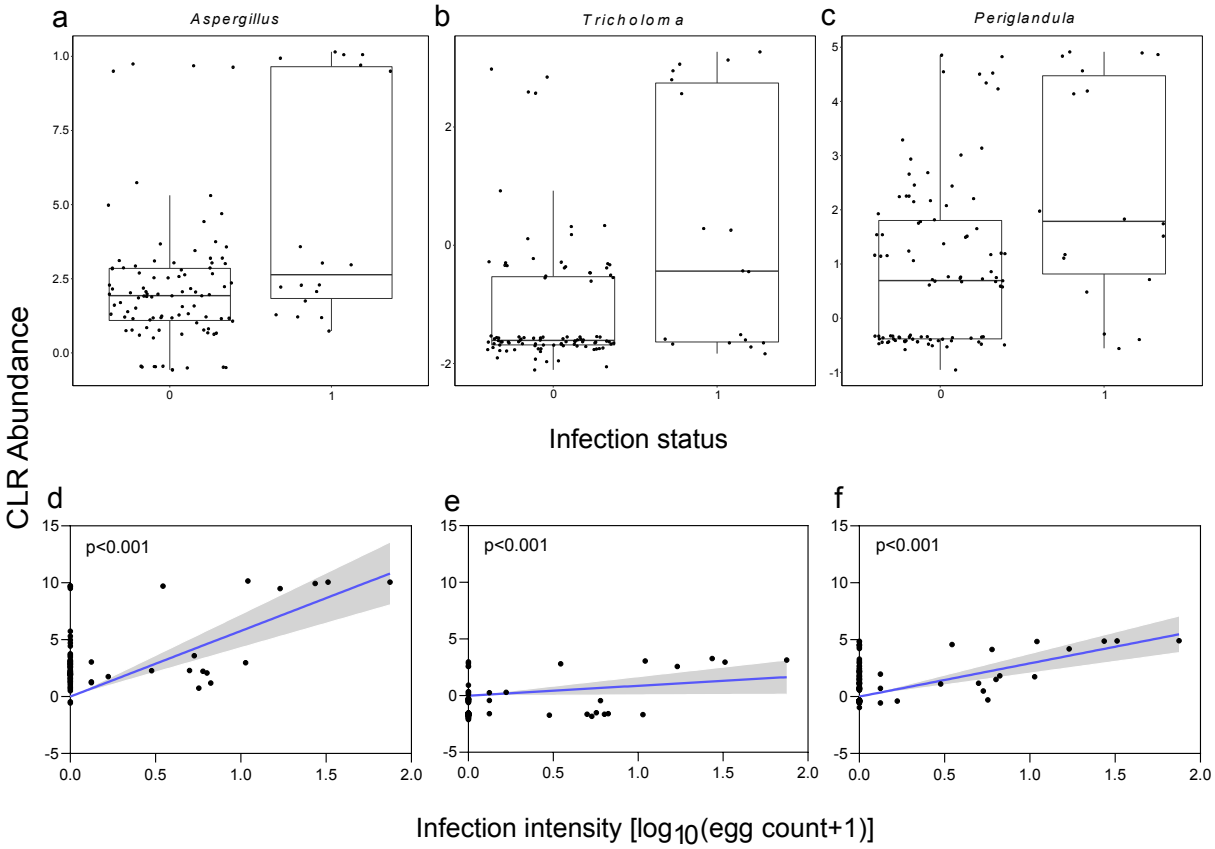

Figure 6.5: Fungi genera whose abundance vary significantly with schistosome infection
Figure a–c) Box plots showing the mean abundance of specific fungi genera, grouped by *S. haematobium* infection status. The horizontal box lines represent the first quartile, the median and the third quartile. Whiskers denote the range of points within the first quartile − 1.5× the interquartile range and the third quartile + 1.5× the interquartile range. Figures d–f) Scatter plots showing linear regression analysis of *S. haematobium* infection intensity and fungi genera abundance. The clr-transformed abundance data was used for all plots. Infection status was coded as 0 and 1 for negative (n=95) and positive (n=18) respectively. *S. haematobium* infection intensity was log transformed [log10 (egg count+1)]. Shaded areas indicate the 95% confidence intervals.

6.5.6 AMR gene characterization

An average 0.06% of read pairs were mapped to AMR genes in the ResFinder database, and 262 AMR genes, belonging to 12 functional drug classes were detected. AMR genes belonging to tetracycline were the most common, followed by beta-lactam, macrolide, sulphonamide and nitroimidazole. Of these, the most abundant genes were *cfxA6*, followed by *tet(Q)*, *tet(W)*, *sul2*, *erm(F)*, and *nimE* (**Figure 6.6**).

Figure 6.6: Overview of antimicrobial resistance (AMR) gene abundance and diversity
From read mapping to the ResFinder database, AMR abundance was calculated for each reference gene across all samples. Stacked bar charts show the most abundant a) AMR gene and b) drug class per sample, proportional to the total AMR within each sample (n=113 biologically independent samples). Charts were generated using gene length-normalised, zero-corrected abundance. "-Others" represents abundance data for all other AMR genes or drug classes in the abundance data set.

6.5.7 Variation in the resistome and association with participant metadata

PCA was used to initially examine variability and to identify clustering according to individual metadata. The model for the first two components explained 18.0% and 48.0% of the total variability in AMR genes and drug classes respectively. Similarly, there was no clustering according to individual metadata. PCA plots and cluster dendrograms of AMR genes and their drug classes per sample is shown in **Supplementary Figures 4–5 in Appendix C**.

PERMANOVA analysis, did not show any significant association of AMR genes with age, village, sex, feeding, malnutrition, stunting, *S. haematobium* infection, previous PZQ

treatment, and antibiotic use. Model summaries of participant metadata and association with AMR genes is shown in **Table 6.2**.

Table 6.2: Model summaries of participant metadata and association with the resistome

Variable	n	p-value	SS-E	SS Total	FDR
Age (years)	113	0.079	179.9	14444.6	0.591
Sex	113	0.847	98.3	14526.3	0.930
Village	113	0.250	149.8	14474.8	0.591
***S. h.* infection status (pos/neg)**	113	0.296	143.1	14481.4	0.591
***S. h.* Infection intensity**	113	0.231	151.7	14472.9	0.591
Malnourished, yes/no (WHZ)	107	0.924	86.0	13909.6	0.930
Stunted, yes/no (HAZ)	109	0.818	102.0	14059.8	0.930
Months breastfed	90	0.880	87.7	11391.0	0.930
Months solid food introduced	102	0.755	107.7	13174.9	0.930
Previous PZQ treatment	105	0.176	158.5	13479.4	0.591
Amoxicillin (yes/no)	76	0.930	89.0	9822.9	0.930
Co-trimoxazole (yes/no)	76	0.247	150.4	9761.5	0.591

Table represents PERMANOVA output for bacteria and fungi genera. Classification of nutritional status was based on a cut off <-2 Z–scores (MOH Malawi 2016). Schistosome infection intensity was log transformed (log10 [egg count+1]). *S. h.*, *S. haematobium*; WHA, weight–for height Z-scores; HAZ, height-for-age Z-scores; pos/neg, positive /negative; raw p, unadjusted p-value; FDR, adjusted p-value (FDR-corrected); SS-E, explained sum of squares; SS Total, total sum of squares.

6.6 Discussion

The human gut microbiome plays a vital role in maintaining overall health. Therefore, changes that occur, especially during establishment of the microbiota in early life, have implications on health and disease risk (Laforest-Lapointe and Arrieta 2017). Understanding the changes that occur in the microbiome and resistome during infection, especially in PSAC, will provide insight into the factors underlying disease progression, differences in disease and treatment patterns, as well as the potential of influencing health through the microbiome in infants and young children. In this chapter, I characterised the structure (abundance and diversity) of the human gut microbiome and resistome in a cohort of Zimbabwean PSAC (≤5 years old). I further test the hypothesis that in PSAC, infection with *S. haematobium* is associated with alterations in the abundance and diversity of the gut microbiota and associated AMR genes. The results showed that in this PSAC population, the microbiota are largely heterogeneous,

and are associated with age and location/geography. This reflects the impact of inherent population-specific characteristics on the structure of the microbiome. *Schistosoma haematobium* infection was associated with significant alterations in the abundance of microbiota. The observed microbiota alterations correlated with infection intensity, strongly supporting that the changes were associated with the presence of *S. haematobium* infection. Evidence of 262 AMR genes belonging to different functional drug classes were detected, but these were not associated with individual-specific metadata, including schistosome infection. Despite the indirect impact that alterations in gut microbiota populations may have on the abundance and diversity of AMR genes, the finding in the current study is in line with suggestions that other population anthropogenetic (of or relating to their origin and development) and socioeconomic factors greatly drive the resistome, and thus have major implications on AMR and health policy (Collignon *et al.,* 2018, Hendriksen *et al.,* 2019).

Age (Yatsunenko *et al.,* 2012, Rodriguez *et al.,* 2015), dietary and environmental patterns (De Filippo *et al.,* 2010, Fan *et al.,* 2014, Martinez *et al.,* 2015), ethnicity, and geography (Deschasaux *et al.,* 2018, Senghor *et al.,* 2018) have an impact on the taxonomic composition of the microbiome. *Prevotella* and *Candida* have been associated with carbohydrate-rich diets (Hoffmann *et al.,* 2013, Gorvitovskaia *et al.,* 2016) and *Bacteroides*, with protein-rich diets (Gorvitovskaia *et al.,* 2016). The presence of these microbial genera in the microbiota is a reflection of the dietary lifestyle among populations in developing countries (De Filippo *et al.,* 2010, Martinez *et al.,* 2015), including infants (Yassour *et al.,* 2016), and these genera were among the most abundant bacteria and fungi genera found in the current study population. Microbiota composition differed significantly across villages, with three genera being more abundant in children from Mupfure than from Chihuri. In addition to evidence on the geographical disparities in microbiota composition (Deschasaux *et al.,* 2018, Senghor *et al.,* 2018), Palmer and colleagues in a longitudinal study showed significant clustering of the microbiota of twins, highlighting the significance of the similarity in microbiota composition

among individuals living in a shared local environment (Palmer *et al.,* 2007). Similar reasons for the observed differences across villages is based on evidence from experimental studies, showing that even with genetically similar families, the microbiota of mice were more similar within households than between households (Ley *et al.,* 2005). Thus, community-specific social, environmental, and lifestyle characteristics may contribute to the observed differences. Similar to findings from previous studies (Kay *et al.,* 2015), there was age but not sex-related associations in bacteria genus abundance and diversity. Given that the population in the current study was ≤5 years old, this is consistent with the microbiome being more dynamic in the early years of life before stabilizing to a more adult-like state (Yatsunenko *et al.,* 2012, Rodriguez *et al.,* 2015).

Differences observed in the microbiome between developing and developed countries have been attributed to factors inherent to developing areas (De Filippo *et al.,* 2010), which may include the role of persistent prevalence of helminth infections, as reviewed by Mishra and colleagues (Mishra *et al.,* 2014). The findings in the current study are consistent with observations that schistosome infection is associated with alterations in the diversity and abundance of specific taxonomic groups in the microbiome (Kay *et al.,* 2015, Schneeberger *et al.,* 2018). In the aforementioned studies, which included preschool and school-aged children, 16S rRNA sequencing showed that *Prevotella* and *Proteobacteria* were more abundant in children infected with *S. haematobium* (Kay *et al.,* 2015) and *S. mansoni* (Schneeberger *et al.,* 2018) respectively, when compared to uninfected children. To the best of my knowledge and at the time of writing this thesis, the novelty of the current study is the fact that it focuses on children 5 years old and below, an age group whose gut microbiome structure is most likely still evolving (Yatsunenko *et al.,* 2012, Rodriguez *et al.,* 2015). The findings presented in this chapter thus provide an important insight into helminth infection and its association with changes during the establishment of the gut microbiome in PSAC in endemic areas. For the

first time, the use of metagenomic sequencing enabled elucidating this association to include the fungal component of the microbiome.

Bacteria and fungi phyla that clearly differentiated the microbiome of schistosome-infected versus uninfected children in the current study were *Proteobacteria*, *Ascomycota* and *Basidiomycota*. These were among the top five most abundant bacteria and fungi and were present in all samples, thus make a major contribution to the overall microbiome composition. *Proteobacteria* has been shown to be present in lower abundances within the microbiota of healthy individuals, and any increases in abundance of members of this phylum confirm dysbiosis and a link with increased disease risk, progression and burden (Shin *et al.,* 2015). Attempts have been made to expand the body of knowledge on the fungi repertoire and diversity in the human microbiome (Gouba *et al.,* 2014) and their association with infection and disease (Mukherjee *et al.,* 2014, Gouba and Drancourt 2015, Mar Rodriguez *et al.,* 2015). However, the role of fungi within the gut microbiota community in health and disease is poorly understood. Studies in mice models have suggested that gut fungal populations directly or indirectly help to maintain healthy intestinal homeostasis, and that dysbiosis has immunological consequences relevant to disease risk progression (Wheeler *et al.,* 2016). For example as observed in the current study, increases in specific fungi populations such as *Aspergillus*, have been associated with increased blood eosinophil levels (Bukelskienė *et al.,* 2006) and an exaggerated T-helper type 2 (Th2) response in mice (Wheeler *et al.,* 2016). The resulting Th2 response is characteristic of schistosome infection, and is important to downregulate host immune responses and to promote parasite survival in the host (Pearce and MacDonald 2002, Colley and Secor 2014). This may explain the observed increases in abundance of specific fungal populations (*Aspergillus*, *Tricholoma* and *Periglandula*) in the schistosome-infected children. However, whether this was due to primary changes in the fungal population or was secondary to changes in the bacterial population is unclear, and further studies into the role of fungal dysbiosis in schistosome infection are warranted.

Although the ability to infer causation was beyond the scope of the current study, it shows that for the microbial genera differentiating the microbiome of the schistosome-infected versus uninfected children, there was a significant positive relationship between microbial abundance and schistosome infection intensity. This strongly supports the fact that the observed changes were associated with the presence of *S. haematobium* infection. However as *S. haematobium* worms mostly reside in the pelvic venous plexus (although some have occasionally been detected in the intestine in Egyptian autopsies (Cheever *et al.,* 1977)), the effect of infection on the diversity of the microbiota is as suggested for intestinal helminths (Broadhurst *et al.,* 2012), but most likely through a more indirect or systemic route than through direct interactions, as discussed earlier in **Chapter 1**. Mishra and colleagues have suggested that the immunomodulatory effects of helminths can extend to the gut microbiota through both direct intestinal interactions and systemic interactions (Mishra *et al.,* 2014). For example, by enhancing the mucosal barrier, tissue repair, production of anti-microbial peptides and reducing dissemination of microbiota to the spleen and liver (Mishra *et al.,* 2014), the upregulation of IL-22 during helminth infection may favour the abundance of specific microbial taxa (Leung and Loke 2013).

Overall, 262 AMR genes were identified, most of which encoded for resistance to tetracycline, beta-lactam, macrolide, and sulphonamide, posing risks to the successful treatment of various conditions including urinary, enteric and respiratory infections (Frank *et al.,* 2007). Amoxicillin (beta-lactam) and co-trimoxazole (sulphonamide) were the most predominant antibiotics reported to have been used among the children in the current study. In addition, ceftriaxone, benzylpenicillin (both beta-lactams), and co-trimoxazole are among the most commonly used antibiotics in Zimbabwe (Zimbabwe National Antimicrobial Resistance Core Group 2017). Given that antibiotic use is selective for AMR in populations (Van De Sande-Bruinsma *et al.,* 2008, Davies and Davies 2010), it is not surprising that majority of the AMR genes identified belonged to these drug classes. However, because the current study did not

determine phenotypic resistance to antibiotics, it is not possible to ascertain that the presence of the AMR genes identified are due to use of these antibiotics within the study population. In addition, the current study did not enable determination of which bacteria species were associated with the AMR genes found, and whether these are encoded on plasmids or on chromosomes. Therefore, with limited studies of such nature in African populations, and given that the current study focused on an apparently healthy population, more research is needed to determine the clinical significance and public health relevance of the AMR genes identified in the current study.

Increased antimicrobial use has a significant effect on the gut microbiota (Bartosch *et al.,* 2004, Dethlefsen *et al.,* 2008), and is selective for AMR in populations (Van De Sande-Bruinsma *et al.,* 2008, Davies and Davies 2010). The limited association of the obtainable antimicrobial use data with both the microbiome and resistome in the current study might be surprising. Dethlefsen and colleagues showed that a majority of the human gut microbiota community that was depleted post-ciprofloxacin administration was restored after 4 weeks (Dethlefsen *et al.,* 2008). The antimicrobial use data obtained in the current study was limited to antibiotic use within the immediate 6 months prior to sampling and was less heterogeneous. Thus, any marked differences in the microbiota abundance from antibiotic use may have been restored before sampling for the current study, and hence missed. The findings shown here are however consistent with those from recent studies on global sewage samples, where compared to antimicrobial use, a much stronger association between AMR genes and socio-economic factors related to health, sanitation, and education was shown (Collignon *et al.,* 2018, Hendriksen *et al.,* 2019). Suggestions are that the influence of multiple factors on AMR genes seems more likely to be the case in low-and middle-income countries, where a high contagion (the spread of resistant strains and genes) between individuals may take place, and any antimicrobial use in one individual may have general effects on the population as a whole

(Pehrsson *et al.,* 2016). Thus antimicrobial use may explain some, but not all the variation in AMR genes in this population (Van De Sande-Bruinsma *et al.,* 2008, Davies and Davies 2010).

In addition, the microbiome is a reservoir for AMR genes (Qin *et al.,* 2010, Hu *et al.,* 2013), and alterations in the gut microbiota composition may impact on AMR gene abundance and diversity. However, suggestions that many inherent population anthropogenetic (of or relating to their origin and development), environmental and socio-economic factors greatly drive the resistome (Collignon *et al.,* 2018, Hendriksen *et al.,* 2019), is in line with the fact that the observed changes in the microbiota was not associated with AMR gene abundance and diversity.

The strength of the current study lies in the fact that metagenomics sequencing to include the fungi component of the microbiome was used, instead of 16S rRNA sequencing, which targets the bacterial component. It also determined the relationship between schistosome infection, the microbiome and resistome, independent of host-related factors including socio-demography, growth and nutritional indices, as well as clinical history. The current study nonetheless also had a few limitations. The cross-sectional study design allowed characterisation of the gut microbiome and its relationship with *S. haematobium* infection at a single time point. A longitudinal study following a natural time-course of schistosome infection and treatment, will inform on the dynamic relationship between schistosome infection and the gut microbiota, and help to elucidate causation and the mechanistic pathways underlying this relationship. A longitudinal study will also inform on any time-course or developmental-related trends in the observed microbiota and AMR gene profiles, and provide an indication of the dynamic features of how they are associated with individual-specific data. Furthermore, relating the presence of AMR genes to measurable phenotypic resistance of bacteria would give a stronger indication of the clinical implications of the AMR genes present.

6.7 Conclusion

In the current study, I characterised the gut microbiome (to include the fungi repertoire) and resistome in a cohort of PSAC (≤5 years old) from Zimbabwe. The results showed that age and location-related differences are associated with abundance and diversity of the gut microbiota. Independent of host-related socio-demographic and clinical factors, there were differences in the gut microbiome but not the resistome, between schistosome-infected and uninfected children, showing largely, an increase in abundance of specific bacteria, and for the first time (as at the time of writing this thesis), fungi genera in infected children. The schistosome-associated alterations in abundance of microbiota populations correlated with infection intensity. Mechanistic studies are required to further explain this relationship. The findings in this chapter suggest microbiome alterations as an additional consequence of schistosome infection, which may be relevant for disease pathogenesis and influence on overall health of the host. It also adds to publicly available data on microbiome and resistome studies from an understudied human population (i.e. PSAC in sub-Saharan Africa).

In the next chapter I discuss the major findings of this thesis in a broader context, their implications for paediatric schistosomiasis research, control and policy, as well as prospects and recommendations.

Chapter 7 General discussion

7.1 Introduction

Schistosomiasis has debilitating consequences in preschool-aged children (PSAC), i.e. children aged 5 years and below. It has been recognised as a disease of public health importance that requires urgent attention in this age group (World Health Organization 2011). Contrary to previously held assumptions that the risk of schistosome infection in PSAC is low (Sacko *et al.,* 2011), there is now a global move to increase research focus on paediatric schistosomiasis to promote disease control and improve child health. This paradigm will be important to achieve the goal of reducing disease-related morbidity and eventually eliminate schistosomiasis (World Health Organization 2020a). However, paediatric schistosomiasis control will only become a priority in endemic countries with limited health resources, when research provides compelling evidence on infection and disease burden and its impact on child health, and when cost-effective intervention tools can be utilised in control programmes.

In this thesis, I address some key research and knowledge gaps in paediatric schistosomiasis, by following a natural time-course of schistosome infections in PSAC living in the Shamva district of Zimbabwe, a schistosome-endemic area (Midzi *et al.,* 2011, Midzi *et al.,* 2014a). I focused on urogenital schistosomiasis, which is caused by *Schistosoma haematobium*, the most common schistosome species occurring in sub-Saharan Africa (McManus *et al.,* 2018, World Health Organization 2020b). The goal was to describe the incidence of the first schistosome infection and morbidity in PSAC, as well as the impact of praziquantel (PZQ) treatment on infection, reversal of morbidity, and reinfection rates. Of particular interest were the early events that occur during the very first schistosome infection and treatment in this age group. The impact of schistosome infection on the host microbiome and metabolism, and how this influences disease and overall health in PSAC was also studied. The thesis presents an integrative approach to schistosomiasis studies in this age group, which contributes to evidence on infection/disease burden and dynamics and the importance of the timing of

diagnosis and treatment in reducing (re)infection and morbidity. In addition, it elucidates the systemic impacts of schistosome infection on the host microbiome, metabolism, and overall health of PSAC.

Here, the major findings in relation to the specific aims outlined in **Chapter 1** are summarised and discussed in broader terms. I also consider the potential contribution of my findings in improving current paediatric schistosomiasis practice and health policy, and suggest how my research could be extended for further benefits.

7.2 Scale of the problem: infection, morbidity and treatment dynamics

In addition to the lack of a child-friendly formulation of PZQ, one of the major reasons for the exclusion of PSAC from schistosome treatment programmes is the lack of compelling evidence on infection, disease and treatment dynamics. Consequently, there is a lack of priority for paediatric schistosomiasis control in most schistosome-endemic areas. The findings from this thesis have demonstrated that PSAC present with schistosome infection (based on egg counts) and morbidity (microhaematuria, malnutrition and stunting) from an early age; the youngest child positive for infection in the study cohort was one year old. Further analysis also showed that the prevalence and intensity of schistosome infection increase as children grow older (see **Chapter 3**). This is in line with previous studies showing that exposure to schistosome infection can occur soon after birth through domestic water activities, and that infection prevalence and intensity is cumulative, increasing with age (Woolhouse *et al.,* 2000, Kanamura *et al.,* 2002, Lengeler *et al.,* 2002, van Dam *et al.,* 2004, Wami *et al.,* 2014). This would suggest that until PSAC receive their first treatment through mass drug administration (MDA), which currently targets school-aged children (SAC; ≥6 years old), infection acquired at an earlier age will continue to accumulate, causing severe, and sometimes, irreversible morbidity.

In schistosome-endemic areas, coinfections and comorbidities influence health and nutrition in children. This makes the identification and interpretation of morbidity associated with schistosome infection challenging. To address this, the proportion of morbidity attributable to schistosome infection was determined (**Chapter 3**). Of the morbidity markers assessed, microhaematuria and stunting were the most dominant markers of schistosome-related morbidity in PSAC; schistosome-positive children were more likely to present with microhaematuria (25 times) and stunting (2 times), compared to uninfected children. In line with the present findings, microhaematuria is associated with *S. haematobium* infection (Wilkins *et al.,* 1979, Gryseels *et al.,* 2006, Colley *et al.,* 2014) and can serve as a point-of-care (POC) field marker of morbidity for urogenital schistosomiasis in PSAC (Salawu and Odaibo 2014, Wami *et al.,* 2015). Studies in older children suggest that the observed effects of schistosome infection on stunting are as a result of chronic parasite-induced inflammation, which persists from infection during childhood (Assis *et al.,* 1998, Friedman *et al.,* 2005, Coutinho *et al.,* 2006). It is however noteworthy that determining the fraction of morbidity attributable to schistosome infection, does not demonstrate causation by schistosome infection. Therefore, the morbidity attributable fraction explains some, but not all the schistosome-related morbidity observed in endemic areas.

Currently, the global infection and disease burden remain unknown in PSAC; the best estimates (up to 50% prevalence) available are from published epidemiological schistosome studies (Woolhouse *et al.,* 2000, Bosompem *et al.,* 2004, Odogwu *et al.,* 2006, Mutapi *et al.,* 2011, Kemal *et al.,* 2019, Mutsaka-Makuvaza *et al.,* 2019, Sacolo-Gwebu *et al.,* 2019). Studies on the incidence of infection and morbidity, in particular, the early events that occur during the very first infection and treatment in PSAC are lacking. To address this, a cohort of previously uninfected children was followed to determine the incidence of the first schistosome infection and morbidity, and the impact of treatment on resolution of early morbidity (**Chapter 3**). Incidence of the first *S. haematobium* infection and morbidity

(i.e. microhaematuria) was recorded every 3 months for a year; the cumulative annual incidence of infection and morbidity was 17.4% and 20.4%, respectively. Therefore even for the very first schistosome infection, PSAC quickly develop clinical symptoms and substantial morbidity as previously suggested (Sacko *et al.,* 2011, King 2015). Furthermore, although morbidity occurred rapidly within 3 months of first infection, this resolved quickly within 3 months of curative treatment with a single standard dose of PZQ (i.e. 40 mg/kg body weight).

Reinfection following curative treatment with PZQ (N'goran *et al.,* 2001, Kabuyaya *et al.,* 2017, Mutsaka-Makuvaza *et al.,* 2018, Woldegerima *et al.,* 2019) has been suggested to occur at a higher rate in PSAC. Reasons are that infection and reinfection might be facilitated by young children staying still in water margins or being bathed in a basin of water collected from infected sources, compared to older children and adults who are more independent and mobile (Stothard *et al.,* 2013). By following a group of previously uninfected children for 2 years, a proof-of-principle study was conducted to compare the effect of a single PZQ treatment on infection and reinfection rates (**Chapter 4**). Results showed that regular quarterly screening and treatment of the first *S. haematobium* infection, reduced the actual time at risk of infection (i.e. the child life-years of infection), and resulted in reduced rates of subsequent new infections. This is consistent with the long-term impacts of antihelminthic treatment in reducing infection transmission (Hodges *et al.,* 2012, Senghor *et al.,* 2016). Furthermore, a single dose PZQ treatment of primary schistosome infections was associated with reduced reinfection rates and intensity a year later. When compared with the reinfection rate observed following the treatment of chronic infections, the rate of reinfection in children who had been treated within 3 months of their first schistosome infection was lower, although not significantly different. These findings are consistent with reports on the impact of PZQ treatment on reduced reinfection rates, as would be the case of treating chronic infections in conventional MDA treatment strategies in schistosome-endemic areas (Wilkins *et al.,* 1987, N'goran *et al.,* 2001). Importantly, my findings show this effect after a single early treatment

in PSAC experiencing their very first schistosome infection. The impact of PZQ treatment on reduced (re)infection rates was also confirmed by the observation that within the first year, the annual rate of first infections was about 3-fold higher, when compared to the annual reinfection rate in previously treated chronically infected children. These observations can be contextualised within the current paradigm that in addition to its direct effects on the parasite, PZQ treatment has an added benefit of inducing/accelerating immune responses associated with protection against reinfection in PSAC (Rujeni *et al.,* 2013), older children, and adults (Watanabe *et al.,* 2007, Black *et al.,* 2010a, Black *et al.,* 2010b, Bourke *et al.,* 2013, Schmiedel *et al.,* 2015).

Evidence presented in this thesis shows that PSAC in schistosome-endemic areas present with significant schistosome infection and morbidity. A significant proportion of the growth/nutrition-related morbidity common in children in these areas is attributable to schistosome infection. In addition, I have demonstrated how quickly the very first schistosome infection can be detected, and when infection-related morbidity develops and can be detected. I have also documented the impact of a single standard dose PZQ treatment in reversing early morbidity and reducing (re)infection rates. Early, regular screening for schistosome infections in PSAC will enhance the ability to detect and treat infections early, while reducing the risk of (re)infection, and preventing severe morbidity.

7.3 Consequences of schistosome infection are systemic and influence overall health of the child

Having determined the incidence of the first schistosome infection in PSAC, the development of early morbidity associated with this infection, and the impact of treatment on morbidity and (re)infection, I then determined the impact of schistosome infection on the host microbiome and metabolism, and how this influences disease and overall health in these children.

Experimental schistosome studies have shown that in order to establish themselves in the host, schistosome worms require essential host-derived hormones, nutrients and cytokines for survival (Amiri *et al.,* 1992, de Mendonca *et al.,* 2000, Davies *et al.,* 2001, Saule *et al.,* 2002, Davies *et al.,* 2004, You *et al.,* 2015). When such essential host factors are not present, there is poor parasite development and fecundity, and infection causes reduced pathology in the host (Cheng *et al.,* 2008, Lamb *et al.,* 2010, Tang *et al.,* 2013). Therefore, a cohort of PSAC who had no evidence of previous infection by schistosomes was followed to their first schistosome infection and curative treatment, and the changes in serum metabolite profiles with infection and treatment were determined (**Chapter 5**). The results demonstrated that the first *S. haematobium* infection is associated with alterations in host metabolites, primarily linked with energy (glycolysis, pentose phosphate pathway, starch, and galactose) and purine metabolism. The observed changes were commensurate with increasing infection intensity and were restored to almost pre-infection levels, following curative treatment with PZQ. A pathway-based model and physiological interpretation indicated that the interplay between the host and schistosome worms is consistent with parasite-related morbidity including malnutrition, poor growth, and poor physical and cognitive performance, common in schistosome-infected children (Freer *et al.,* 2018). The observed metabolite alterations were also consistent with the hypothesis that schistosome worms have the ability to manipulate the host metabolic system for molecules such as lipids (Meyer *et al.,* 1970, Brouwers *et al.,* 1997) and purines (Levy and Read 1975b), essential for their survival in the host. Studies of *S. mansoni* infection have reported similar findings in terms of alterations in energy and liver metabolism, in addition to gut microbiota metabolism (Balog *et al.,* 2011, Panic *et al.,* 2018); in these studies however, cohorts were not limited to PSAC. Together, my observations are in line with findings from studies conducted in experimental models of schistosome infection (Ahmed and Gad 1995, Wang *et al.,* 2004, Liu *et al.,* 2019), showing that such host metabolic alterations can begin as early as three weeks post-infection, and are essential for parasite development and disease progression (Wu *et al.,* 2010b).

Given the vital role the human gut microbiome plays in maintaining overall health (discussed in **Chapter 1**) (Human Microbiome Project Consortium 2012), alterations in the gut microbiota population that may occur as a result of schistosome infection, have implications for disease susceptibility (Laforest-Lapointe and Arrieta 2017). In addition, the microbiome is a reservoir for antimicrobial resistance (AMR) genes (Qin *et al.,* 2010, Hu *et al.,* 2013), hence, it is possible that alterations in the gut microbiota could have an impact on AMR gene abundance and diversity. Hence, I characterised the structure (abundance and diversity) of the gut microbiome and resistome in a cohort of PSAC, and tested the hypothesis that infection with *S. haematobium* is associated with alterations in the abundance and diversity of the gut microbiota and associated AMR genes (**Chapter 6**).

Independent of host-related factors including socio-demography, clinical history, and growth and nutritional indices, *S. haematobium* infection was associated with significant alterations in the abundance of specific gut microbiota populations; there was predominantly an increased abundance of bacterial and fungal phyla from *Proteobacteria*, *Ascomycota* and *Basidiomycota* in schistosome-infected children, compared to uninfected children. The observed microbiota alterations correlated with infection intensity, strongly supporting that the changes were associated with the presence of *S. haematobium* infection. Recently, differences in microbiota abundance between infected and uninfected children was reported during infection with *S. haematobium* (Kay *et al.,* 2015, Ajibola *et al.,* 2019) and *S. mansoni* (Schneeberger *et al.,* 2018). These studies included both PSAC and older children (ages 6 months to 15 years), whilst my findings focus on PSAC. Further analysis showed that individual-specific metadata, including schistosome infection, were not associated with the resistome in this cohort of PSAC. This may be explained by suggestions that many inherent population anthropogenetic (of or relating to their origin and development) and environmental factors greatly influence the resistome (Collignon *et al.,* 2018, Hendriksen *et al.,* 2019).

Much of our understanding of the schistosome–metabolism–microbiome interactions in humans has been facilitated by experimental studies (discussed in **Chapter 1**). For the work presented here, the analysis of matching pre- and post-infection samples in a cohort of PSAC with no infection history, allows for an understanding into the early metabolic responses that contribute to disease progression and morbidity from the first schistosome infection in PSAC. In addition, the gut microbial population in very young children continues to evolve until about age 3–5 years (Yatsunenko *et al.,* 2012, Rodriguez *et al.,* 2015). Thus, my findings provide understanding into the association between helminth infection and the changes that occur during the establishment of the gut microbiome in young children (i.e. ≤5 years old). It further suggests microbiome alterations as an additional consequence of schistosome infection, which may be relevant for disease pathogenesis and influence on overall health of the host. As our knowledge in the field of the schistosome–metabolism–microbiota interactions in humans expand, further phenotypic and mechanistic studies will contribute to a better understanding of the association between metabolic/microbiome disturbances and the aetiology of schistosome-related pathology in children.

7.4 Implications of findings for improving paediatric schistosomiasis control and health policy

There is now an increased recognition of the health consequences of schistosomiasis in PSAC, and concerted efforts are being made to address research and knowledge gaps, while ensuring access to treatment for PSAC in different endemicity settings. In this thesis, I was able to demonstrate the dynamics of schistosome infection and treatment in PSAC, using cross-sectional and longitudinal field studies conducted in a schistosome-endemic area in Zimbabwe. In schistosome-endemic areas, preventive chemotherapy programmes conducted through MDA target SAC (WHO Expert Committee 2002, World Health Organization 2006). Unlike SAC, PSAC are not accessible in schools (although some may be in early child development centres) and accessing them for diagnosis and treatment is a challenge. Similar

to studies conducted in PSAC in schistosome-endemic areas (Mutapi *et al.,* 2011, Mutsaka-Makuvaza *et al.,* 2018), and as recommended by the World Health Organization (WHO) (World Health Organization 2011), the work presented in this thesis demonstrates the utility of primary health care centres and current health intervention programmes such as Child Health Days and the Expanded Programme on Immunization (EPI), for the regular screening and treatment of schistosomiasis in PSAC. This suggests that pending the roll-out of the paediatric PZQ formulation for the treatment of schistosomiasis in PSAC through MDA programmes, access to treatment can be extended to include PSAC via primary health centres on a case-by-case basis (World Health Organization 2011, Bustinduy *et al.,* 2016a). To eliminate schistosomiasis as a public health problem (currently defined as <1% of heavy intensity schistosomiasis infections), one of the critical action points highlighted in the recently published WHO neglected tropical diseases (NTD) roadmap for 2021–2030, is to extend treatment to all at-risk populations using more cost-effective strategies (World Health Organization 2020a). Thus, the use of primary health care centres to access and treat PSAC for schistosomiasis, as demonstrated in this thesis, presents an opportunity to extend treatment to PSAC who are excluded from treatment in most endemic countries.

Determining the proportion of morbidity attributable to schistosome infection will make the identification and interpretation of schistosome-related morbidity easier; my findings highlight the role or relevance of urinary morbidity markers and anthropometric measures, as a tool for identifying schistosome-related morbidity in endemic areas. For example previous findings from Zimbabwe (Wami *et al.,* 2015) and Nigeria (Salawu and Odaibo 2014) have demonstrated the significance of microhaematuria as a POC field marker of morbidity for urogenital schistosomiasis in PSAC. Chronic growth/nutrition-related morbidity indices such as stunting can also help identify high-risk groups, and measure the health impacts of infection and treatment in schistosome-endemic areas. My work further demonstrates that microhaematuria coincides with schistosome infection and is resolved with curative treatment;

this highlights the significance of microhaematuria in post-treatment monitoring of PSAC, as applied during large-scale chemotherapy for schistosomiasis in SAC (Koukounari *et al.,* 2007, Webster *et al.,* 2009, King and Bertsch 2013).

As demonstrated in this thesis, a regular screening strategy to detect and treat schistosome infections early in PSAC, is essential to avert irreversible morbidity; this can be done using the currently available diagnostic, morbidity and treatment tools. Schistosome infection and morbidity can be detected early in PSAC using parasitology (egg counts) and dipstick techniques (microhaematuria) within 3 months of first infection, and morbidity resolves 3 months after curative treatment with PZQ. There are ongoing phase III clinical trials for the leading paediatric PZQ formulation in countries including the Ivory Coast and Kenya (target age is 3 months–6 years) (Paediatric Praziquantel Consortium 2020). Preparations are also far advanced to conduct a similar trial in Zimbabwe, in which our research group is involved. While we await promising results from these trials, the findings reported here will have a great impact in planning and policy development for schistosomiasis control in endemic areas, in terms of determining the best treatment strategy to reduce subsequent new infections, reinfections, and long-term morbidity. For example, given the health consequences in terms of long-term morbidity from chronic schistosome infections, a routine screen-and-treat strategy as part of routine child health and development monitoring activities will optimise the chances of detecting and treating infections early, while reducing the risk of new infections, reinfection, and severe morbidity.

The evidence presented on the effects of schistosome infection on the host microbiome and metabolism will have a major impact on helminth infection control in endemic areas. First, it adds to the repository of limited information on how the human gut microbiome and metabolism influence schistosome-related disease and morbidity in PSAC. Secondly, it contributes to our understanding on the factors underlying disease progression, differences in disease patterns, as well as the development of new strategies targeted at reducing

schistosome-associated morbidity in infants and young children. For instance, it will provide a basis for understanding the role of interventions such as nutraceuticals, in influencing health through the microbiome and enhanced nutrition/metabolism for selected helminth infections.

It is also worth mentioning that after adoption of the Global Action Plan on antimicrobial resistance by the 68th session of the World Health Assembly in 2015 (Geneva, Switzerland), Zimbabwe put in place a framework to develop an AMR National Action Plan through a situational analysis of antimicrobial use and resistance in the country (Zimbabwe National Antimicrobial Resistance Core Group 2017). The work presented in this thesis in relation to characterising the resistome of this preschool population has already contributed to the development and launch of a One Health Antimicrobial Resistance National Action Plan for 2017–2021 in Zimbabwe (https://www.ed.ac.uk/files/atoms/files/zimbabwe_nap_2_1.pdf). In keeping with calls from the WHO and the United Nations on strategic policies on global antibiotic use, scientific information comparable to the data provided in this thesis will help formulate policy and guide revisions in antibiotic use in the country, and for the rest of sub-Saharan Africa.

My findings together contribute to identifying appropriate morbidity indicators, alternative approaches for utilizing current interventions for maximum health benefits, developing new interventions for schistosomiasis control, and informing policy to improve infection and disease control. This is in line with the critical action points highlighted for schistosomiasis control and elimination in the new WHO NTD roadmap for 2021–2030 (World Health Organization 2020a). These action points are discussed in further detail in the subsequent section.

7.5 Treating paediatric schistosomiasis contributes to disease elimination and meeting the SDG targets

The recently updated WHO NTD roadmap for 2021–2030 highlights three critical action points for eliminating schistosomiasis as a public health problem, i.e. <1% of heavy intensity schistosomiasis infections (World Health Organization 2020a). These include defining indictors of morbidity, extending treatment to those in need and ensuring access to medications, as well as developing new interventions for schistosomiasis control. Thus, the work and evidence presented here contributes to realising these goals, especially for improving paediatric schistosomiasis control, by demonstrating:

(i) Evidence on the early events that occur during the first schistosome infection in PSAC in endemic areas.

(ii) The utility of current health systems and tools for extending treatment to PSAC.

(iii) The best approach and timing to access PSAC for treatment to reduce reinfection and morbidity.

(iv) The utility of growth and nutritional indices as indicators of schistosome-related morbidity and identification of high-risk groups.

(v) Insight into the development of new interventions targeted at reducing morbidity, including the role of nutraceuticals for influencing health and improving nutrition-related morbidity in schistosome-infected children.

Extending the findings presented in this thesis to conduct further studies across different endemicity settings, will contribute to scientific evidence appropriate for improving disease control and eventually eliminate the disease in schistosome-endemic countries.

Providing such compelling evidence (as demonstrated in this thesis) will help to ensure that paediatric schistosomiasis control becomes a priority in endemic countries with limited health resources. This will be important for achieving the Sustainable Development Goals (SDGs)

for health (i.e. Goal 3; ensure healthy lives and promote well-being for all at all ages). As part of this goal, NTDs are highlighted in target 3.3, which calls for ending the epidemic of NTDs by 2030. Thus, successful interventions against NTDs such as schistosomiasis could have an impact on and improve prospects for achieving the SDG targets and vice versa. For example, improving the health of children through improved paediatric schistosomiasis interventions will mean enabling children to have access to education and with good physical and cognitive performance (Goal 4), alleviating hunger by ensuring children make the most of their nutrition, free of infections (Goal 2), and reducing long-term morbidity for a productive future life and reduction of poverty (Goal 1). Conversely, achieving other SDGs will also impact positively on the control of schistosomiasis and other NTDs. For instance the provision of adequate water, sanitation and hygiene (WASH) (Goal 6) is a complementary schistosomiasis control method, whilst the provision of resilient infrastructure (Goal 9) will improve health care delivery, and promote a strong logistic chain for the delivery of treatment and essential resources to the remotest of endemic areas.

Finally, in line with the public health measures in place for the recent COVID-19 pandemic, particularly physical distancing, the WHO in April 2020 recommended that all community-based surveys and MDA activities be suspended until further notice (World Health Organization and the United Nations Children's Fund (UNICEF) 2020). This will impact negatively on the current progress for schistosomiasis control, logistics and resource supply, the ongoing phase III clinical trials for the roll-out of the paediatric PZQ, and overall, the elimination targets set for 2030. Therefore, while the evidence presented here is important to improving schistosomiasis control, there is a need to examine the potential impact of the suspension/delay of MDA, the use of primary health facilities for diagnosis and treatment, as well as the utility of complementary measures such as WASH, education, and vector control measures in this scenario. This is essential to propose concrete mitigation approaches that will serve as a basis for providing remedial strategies in the context of scenarios of the current and

future pandemics of any kind. Modelling analysis currently being conducted by the Expanded Special Project for Elimination of Neglected Tropical Disease (ESPEN) will be a good starting point (see https://espen.afro.who.int/updates-events/covid-19).

7.6 Prospects and recommendations

The work presented in this thesis was conducted to answer a series of epidemiological, molecular and biochemical questions relating to schistosome infection and disease dynamics (aims outlined in **Chapter 1**). The main schistosome diagnostic method applied to answer these questions was the recommended conventional field-applicable parasitological methods, i.e. the urine filtration and Kato-Katz techniques (World Health Organization 2020b). There are operational challenges of sampling and diagnostic sensitivity associated with the current parasitological diagnostic methods (Bergquist *et al.,* 2009, Coulibaly *et al.,* 2013, Wami *et al.,* 2014, Utzinger *et al.,* 2015). Schistosome infection in PSAC usually presents as low intensity infections, which may be missed by parasitological techniques, thus underestimating the prevalence of schistosome infection (Le and Hsieh 2017). However, there is lack of a more convenient, specific, rapid, and cheap alternative diagnostic method that is suitable for field applications in schistosome-endemic areas. Realistically looking ahead to primary health care for infants and children in schistosome-endemic areas, there is a need for further research into more sensitive rapid POC tests that integrate diagnosis for all *Schistosoma* species infections, suitable for large scale field applications. However, the use of parasitological methods for infection diagnosis in all aspects of this thesis allows to compare the present findings to other studies, and to inform developing targeted interventions, while parasitological methods remain the predominant schistosome diagnostic in PSAC in schistosome-endemic areas.

Longitudinal studies across different epidemiological and endemicity settings are needed to evaluate the access, screening and treatment strategy used in this thesis. These should also include determining the immunological mechanisms underlying the resistance to reinfection associated with early treatment of schistosome infections in PSAC. For instance, it will be

imperative to determine the levels of various immunological markers, before and after treatment at the time of measuring reinfection rates. These could include T-helper type (Th)1 cytokines such as interferon gamma, IL-1, IL-2, and IL-6, Th2 cytokines such as IL-4, IL-5, IL-10, and IL-13, as well as parsite-specific IgE and IgG antibody classes. There is also a need for larger studies to determine the practical long-term operational and economic implications of regular screening to diagnose and treat primary schistosome infections of all species in PSAC. Such studies will be important in contributing evidence to determine the best approach and timing to access PSAC for chemotherapy, and to reduce reinfection and long-term morbidity across a diversity of endemicity settings. In addition, the utility of growth and nutritional indices as indicators of schistosome-related morbidity will need to be included in such evaluations, to ascertain their utility as a tool for identification of high-risk groups, and to monitor morbidity. Given that several factors including dietary habits and socio-economic status can influence the growth and nutritional sequelae observed in children in schistosome-endemic areas, studies which can separate the effects of schistosome infections from other confounders would be informative in identifying and apportioning causation for schistosome-related morbidity attributable fractions.

Metabolic alterations in response to schistosome infection, in conjunction with the metabolic pathway-based analysis and interpretation was described in this cohort of PSAC. This gave insight into the host-pathogen interactions that lead to establishment/survival of the parasite, and consequently, disease and morbidity, early in the first schistosome infection. However, long-term studies relating measurable clinical manifestations of schistosome infection in children to such metabolic alterations, would give a stronger indication of the clinical implications of the schistosome-induced metabolic disturbances. For instance, it will be important to conduct studies following a natural time-course of schistosome infection and treatment, which will measure metabolic profiles in PSAC in addition to developmental time-course profiles of growth, nutrition, physical activity and cognition.

The findings on the effects of schistosome infection on the microbiome indicate that phenotypic and mechanistic studies to understand the systemic interactions between schistosomes and the microbiome, and to elucidate causation in natural human infections are needed. Microbiome research studies that focus on PSAC, will provide further insight for developing specific interventions for influencing health through the microbiome in infants and young children (Robertson *et al.,* 2019). Longitudinal studies tracking individuals over a long-term after curative treatment (preferably at regular time point intervals), to ascertain whether and how quickly their microbiota returns to an uninfected state are recommended. Such studies will also help to clarify the causality of the observed changes, and the effect of curative treatment on reversal of morbidity associated with alterations in microbiota. Human microbiome studies that include characterising the resistome will also need to include relating the presence of AMR genes to measurable phenotypic resistance to bacteria, and what the clinical implications are.

The studies on the association between schistosome infection and the host microbiome and metabolism were limited by sample size. This was especially so for the investigation on the effects of schistosome infection on the host metabolism, where following a natural time-course of first schistosome infections, meant that there was no control over the number of infected or uninfected children. Much of our understanding of the association between schistosome infection and the host microbiome and metabolism are from experimental studies (as described in **Chapter 1**). In addition, there are limited schistosome–microbiome and schistosome–metabolism studies focusing solely on PSAC, to inform sample sizes for the work conducted in this thesis. Nonetheless, the current evidence available from studies including older individuals [for metabolomics; (Balog *et al.,* 2011, Panic *et al.,* 2018), and for microbiome studies (Kay *et al.,* 2015, Schneeberger *et al.,* 2018, Ajibola *et al.,* 2019)], show that the sample sizes used were sufficient to detect significant differences. The findings indicate that it is important to extend these studies to larger preschool cohorts.

7.7 Final conclusions

Based on the work described, the conclusions are as follows:

(i) The results demonstrate for the first time, incidence of the first *S. haematobium* infection and morbidity in PSAC, as estimated by egg counts and microhaematuria respectively. This infection and morbidity occur rapidly, can be detected within 3 months of first infection using currently available tools, and morbidity resolves within 3 months of curative treatment with PZQ. Chronic growth and nutrition-related morbidity such as stunting and malnutrition are attributable to *S. haematobium* infection in PSAC, and can help identify high-risk groups, to quantify and monitor morbidity in PSAC. Undelayed screening and treatment of schistosome infections in PSAC is essential to avert accumulative morbidity, which can affect overall health.

(ii) PZQ treatment of the first schistosome infection in PSAC, is associated with resistance to reinfection. Regular quarterly screening and treatment of the first *S. haematobium* infection results in reduced rates of subsequent new infections. A routine screen-and-treat strategy as part of routine child health and development monitoring activities will optimise the chances of detecting and treating infections early, while reducing the risk of new infections, reinfection, as well as severe morbidity.

(iii) Early in the first *S. haematobium* infection, there are significant alterations in host metabolite profiles, primarily related to energy and purine metabolism. These changes correlated with infection intensity and resolved 12 weeks post-curative antihelminthic treatment. The findings suggest an interplay between the host and schistosome worms, consistent with parasite survival, disease progression, and parasite-related morbidity including malnutrition, poor growth, and poor physical and cognitive performance.

(iv) There are significant differences in the gut microbiome but not the resistome, between schistosome-infected and uninfected PSAC; there is largely an increase in abundance of specific bacterial and fungal microbiota in infected children. The observed alterations in microbiota abundance correlated with infection intensity. This suggests microbiome alterations as an additional consequence of schistosome infection, which may be relevant for disease pathogenesis.

This thesis provides an overview of the current knowledge and research in paediatric schistosomiasis practise, while identifying and addressing important research and knowledge gaps. Original research was conducted to answer important questions relating to schistosome infection and disease dynamics, and the health impacts of infection and treatment, during the first infection event in PSAC (i.e. ≤5 years old). This thesis also answers questions on the biochemical and molecular mechanisms underlying disease and treatment, as they relate to the metabolic system and the gut microbiome in this age group. Taken together, the findings contribute to bridging the knowledge gaps relevant to deliver sustainable control of schistosomiasis in PSAC, to inform development of specific interventions targeted at reducing morbidity in this age group, and to strengthen elimination programmes and improve overall health.

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Appendix A Supplementary information–Chapter 3

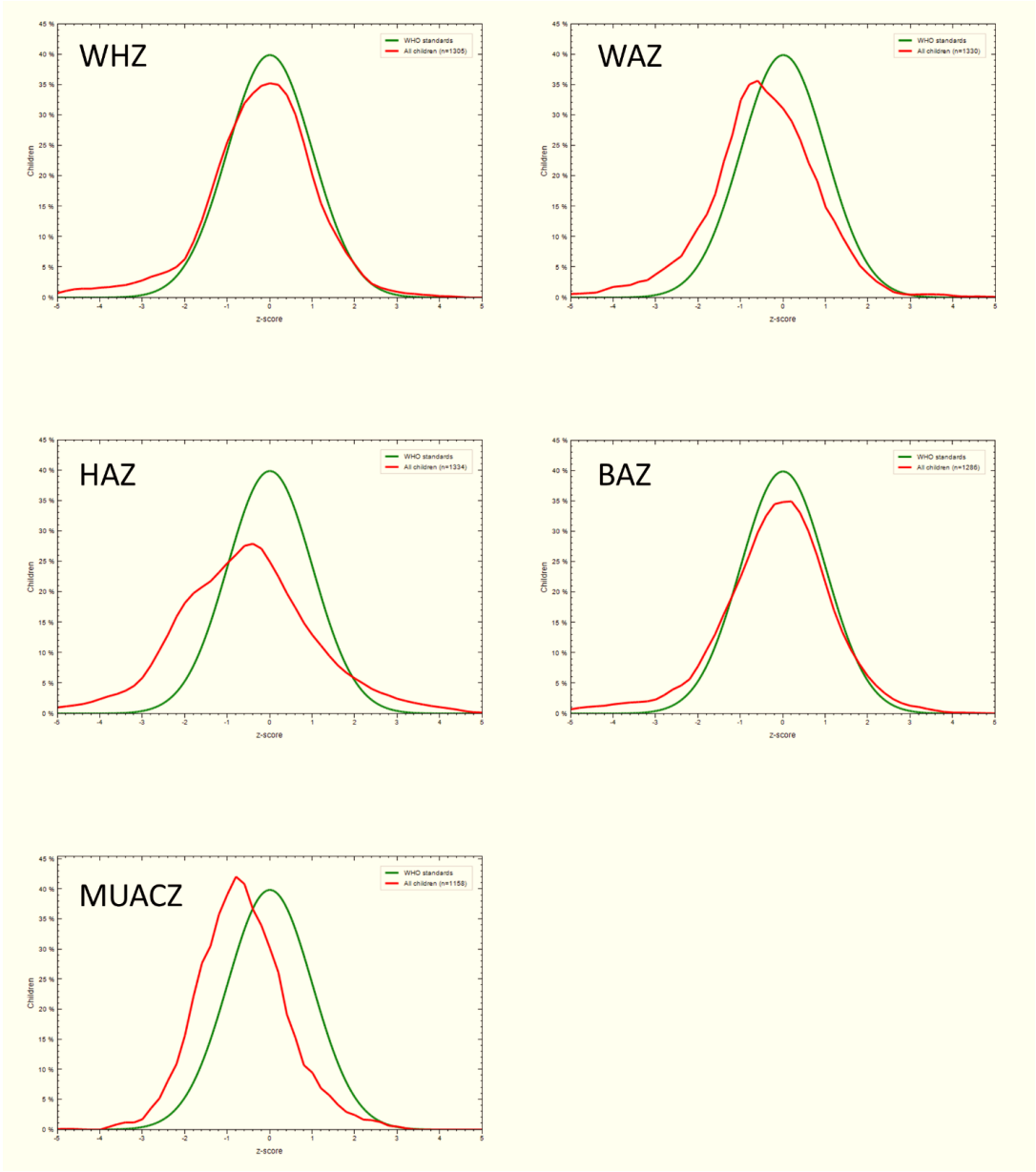


Figure 0.1: Output from growth and nutrition analysis of the study population at baseline. Output was generated from the World Health Organization (WHO) Anthro software, version 3.0.1 (http://www.who.int/childgrowth/en/). The tool, based on raw anthropometric (weight, height) and demographic data (age and sex), generates anthropometric estimates and their corresponding Z-scores, based on four indicators; length/height–for–age, weight–for–age, weight–for–length/height, and body mass index (BMI)–for–age. All values are based on WHO standards (Red) and plots are compared to the WHO standard population curve (Green). HAZ, height–for–age Z-scores; WAZ, weight–for–age Z-scores; BAZ, BMI–for–age Z-scores (BAZ), MUACZ, Mid-upper arm circumference Z-scores; WHZ, weight–for–height. Measures below the median, based on indicators were defined as Z-scores < -2.

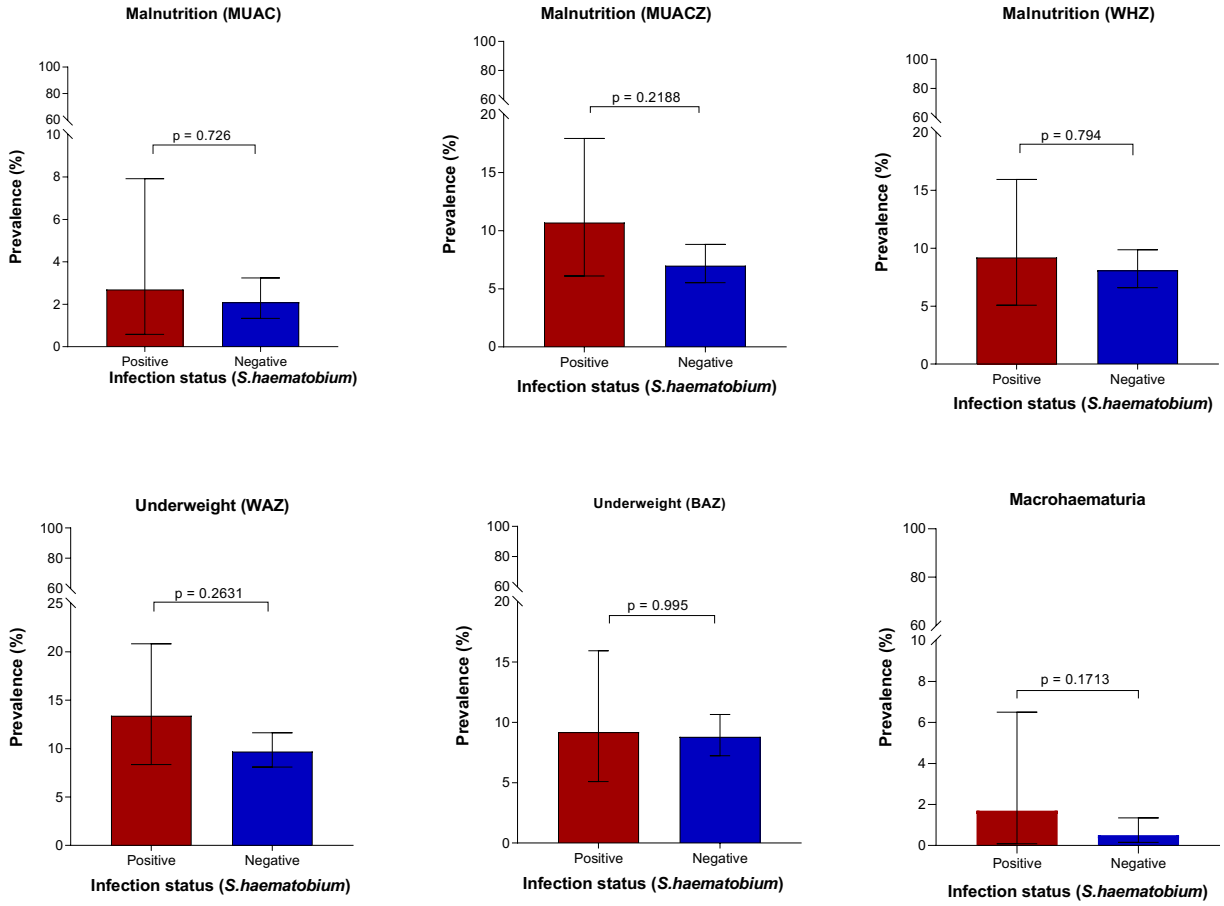


Figure 0.2: Schistosome-related morbidity at baseline, stratified by schistosome infection status. With the exception of stunting and microhaematuria (shown in chapter 3), all other indicators were not significantly different between groups, although slightly higher amongst *S. haematobium*-positive children. Error bars indicate 95% confidence intervals of the observed prevalence. p-values test for the differences in prevalence between morbidity for each infection status (Fisher's exact test).

Appendix B Supplementary information–Chapter 5

Table 0.1: Important metabolites identified by fold change analysis by sex.

Metabolite	Fold Change (FC)	$\log_2$ (FC)
3-Phosphoglyceric acid	0.48062	-1.057
β-Alanine	0.46924	-1.0916
cis-Aconitic acid	0.45987	-1.1207
Sarcosine	0.44656	-1.1631
Ribulose-5-phosphate	0.32765	-1.6098
Citrulline	0.28031	-1.8349
Spermidine	0.25941	-1.9467
Inosine	0.25062	-1.9965
Uracil	0.18962	-2.3988
Glyoxylic acid	0.15585	-2.6817
Guanine	0.10453	-3.258
Uridine diphosphate	0.10453	-3.258
Creatinine	0.10332	-3.2748
2-Phosphoglyceric acid	0.072414	-3.7876
Fumaric acid	0.058175	-4.1035
Glucose-1-phosphate	0.043478	-4.5236

Table shows metabolites ranked in order of decreasing absolute fold change. Analysis is according to female/male ratio.

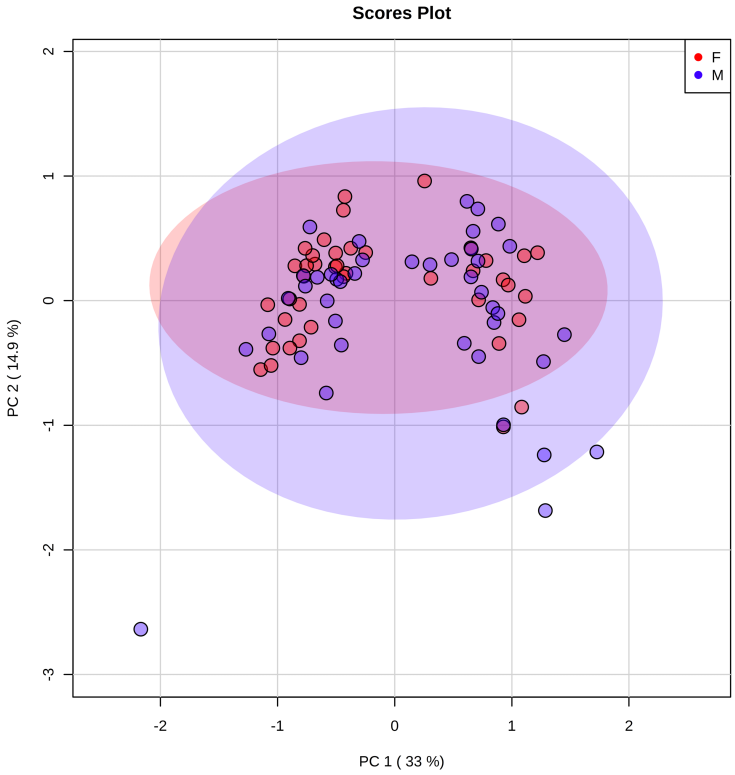
Table 0.2: Correlation pattern analysis of metabolites showing a female to male pattern

Metabolite	Correlation	t-statistic	p-value	FDR
Creatinine	0.50335	5.2427	1.23E-06	7.90E-05
Citrulline	0.46873	4.7757	7.85E-06	0.00025121
cis-Aconitic acid	0.31171	2.9525	0.0041229	0.087955
gamma-aminobutyric acid (GABA)	0.2977	2.8066	0.0062697	0.10031
Sarcosine	0.26854	2.509	0.014102	0.1805
2-Oxoisovaleric acid	0.2305	2.1319	0.036047	0.36727
Isocitric acid	0.22574	2.0855	0.040171	0.36727
Glucose-1-phosphate	0.21615	1.9925	0.049688	0.3975
3-Phosphoglyceric acid	0.18689	1.7122	0.090693	0.60492
Asparagine	-0.18475	-1.6918	0.094519	0.60492
Ribulose-5-phosphate	0.17364	1.5868	0.11644	0.67749
Fumaric acid	0.16667	1.5213	0.13207	0.70437
β-Alanine	0.14805	1.3473	0.18164	0.77685
Inosine	0.14542	1.3228	0.18961	0.77685
Guanosine monophosphate (GMP)	0.14044	1.2766	0.2054	0.77685
2-Phosphoglyceric.acid	0.14004	1.2729	0.20669	0.77685
Glyoxylic acid	0.13052	1.1848	0.23956	0.77685
Inosine monophosphate (IMP)	0.1264	1.1468	0.25483	0.77685
Pyruvic acid	0.12088	1.0959	0.27636	0.77685
Citric acid	0.11845	1.0737	0.28617	0.77685
N.N-Dimethylglycine	0.11823	1.0716	0.28709	0.77685
Glucose-6-phosphate	0.10428	0.94363	0.34817	0.77685
3-Hydroxybutyric acid	0.10402	0.94127	0.34936	0.77685
Guanine	0.10342	0.93577	0.35217	0.77685
Uridine diphosphate (UDP)	0.10342	0.93577	0.35217	0.77685
Adenosine monophosphate (AMP)	0.10328	0.93453	0.35281	0.77685
Hydroxyproline	0.10234	0.92596	0.35722	0.77685
Uracil	0.095602	0.86438	0.38993	0.77685
Methionine	0.095524	0.86367	0.39032	0.77685
Arginine	-0.095024	-0.8591	0.39282	0.77685
Lysine	-0.093629	-0.84638	0.39984	0.77685
Spermidine	0.092362	0.83483	0.40627	0.77685
Glutamine	0.090823	0.8208	0.41417	0.77685
Aspartic acid	-0.090674	-0.81944	0.41494	0.77685
Phenylalanine	-0.086671	-0.78298	0.43592	0.77685
Hypoxanthine	-0.086471	-0.78117	0.43698	0.77685
Malic acid	0.079649	0.71913	0.47413	0.82012
Betaine	-0.075391	-0.68045	0.49816	0.839
Isoleucine	-0.067526	-0.60912	0.54415	0.89296
Succinic acid	-0.063331	-0.57113	0.56949	0.90425
Ethanolamine phosphate	0.060998	0.55	0.58383	0.90425
Creatine	-0.057916	-0.52212	0.60301	0.90425

Metabolite	Correlation	t-statistic	p-value	FDR
Histidine	0.055047	0.49617	0.62112	0.90425
Lactic acid	0.049938	0.45001	0.65391	0.90425
2-Oxoglutaric acid	0.048837	0.44006	0.66106	0.90425
Uridine	-0.04522	-0.4074	0.68479	0.90425
Carnosine	0.043415	0.3911	0.69675	0.90425
2-Hydroxybutyric acid	0.041292	0.37195	0.7109	0.90425
Homoserine	0.039607	0.35675	0.72221	0.90425
Leucine	-0.038557	-0.34727	0.72929	0.90425
Tryptophan	0.037895	0.3413	0.73376	0.90425
Gluconic acid	0.036951	0.33279	0.74015	0.90425
Ornithine	0.035674	0.32127	0.74883	0.90425
Alanine	0.031103	0.28006	0.78014	0.91498
Choline	-0.030209	-0.272	0.78631	0.91498
Serine	-0.025848	-0.23271	0.81657	0.93323
Glutamic acid	-0.019584	-0.17629	0.86051	0.95323
Proline	-0.019108	-0.172	0.86386	0.95323
Adenosine diphosphate (ADP)	-0.011021	-0.099196	0.92123	0.99315
Valine	-0.0084686	-0.07622	0.93943	0.99315
Tyrosine	-0.0074647	-0.067185	0.9466	0.99315
Glycine	0.0043594	0.039235	0.9688	0.99402
Guanosine diphosphate (GDP)	0.0013403	0.012063	0.99041	0.99402
Threonine	-0.00083565	-0.0075209	0.99402	0.99402

Table shows metabolites ranked in order of decreasing significance based on absolute FDR values. FDR, adjusted p-value (False discovery rate corrected).

a)



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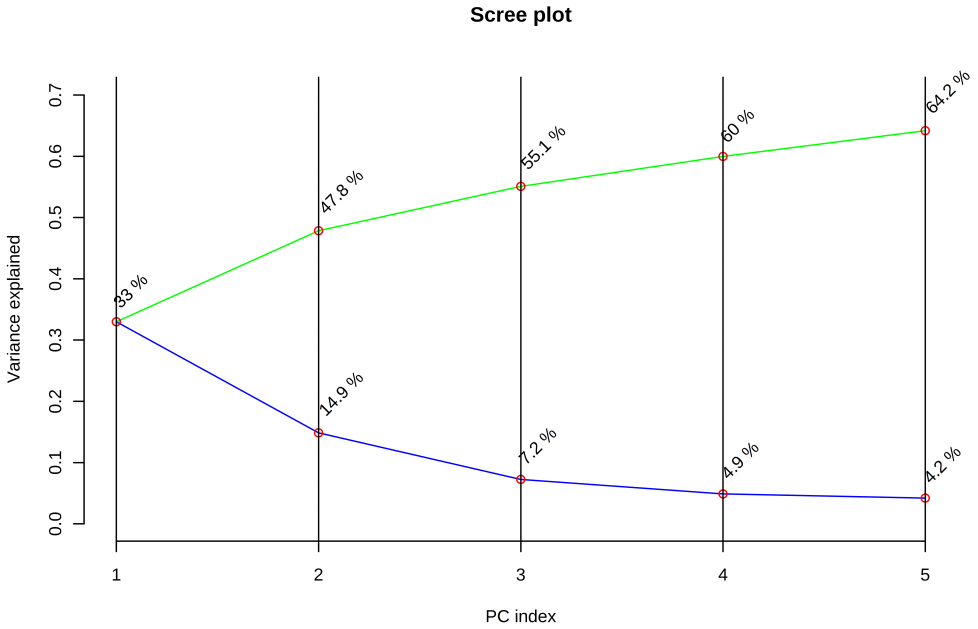
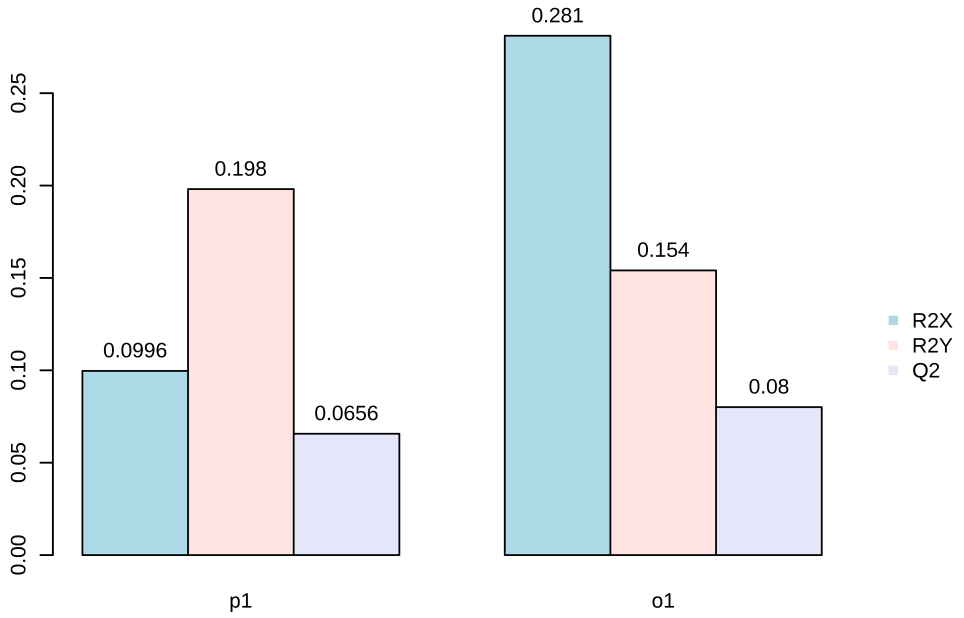


Figure 0.1: Principal component analysis (PCA) of metabolite features by sex at baseline.
a) Scores plot between the selected principal components. The explained variances are shown in brackets. b) Scree plot shows the variance explained by principal components. The green line on top shows the accumulated variance explained; the blue line underneath shows the variance explained by individual principal components.

a)



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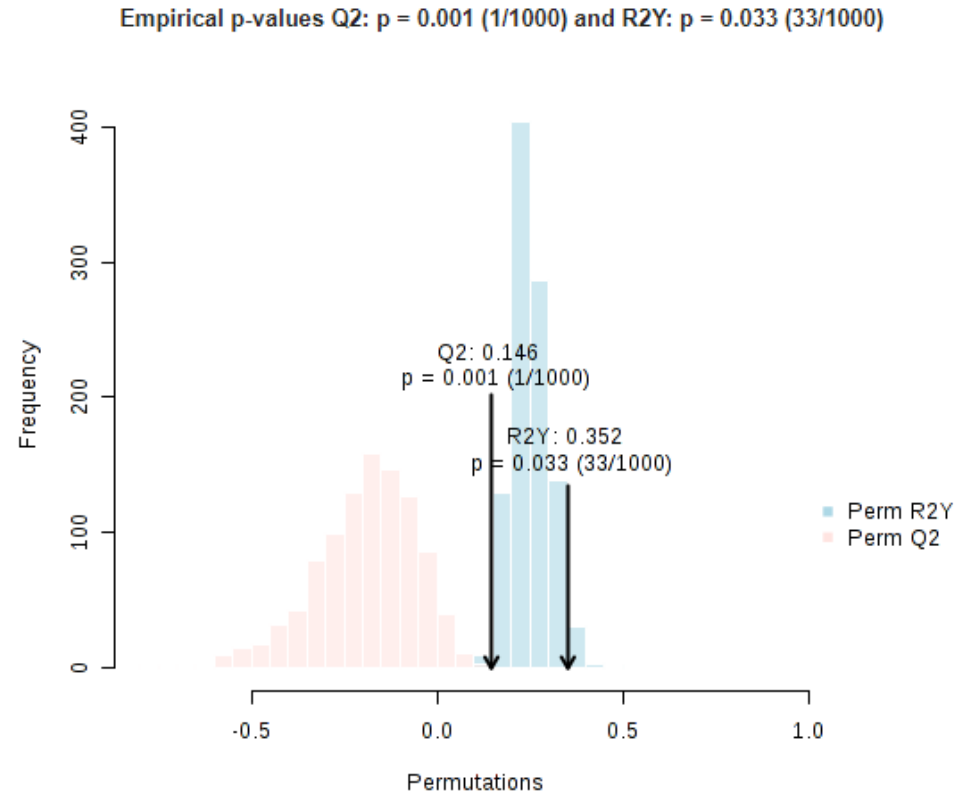


Figure 0.2: Orthogonal Projections to Latent Structures Discriminant Analysis (OPLSDA) model statistics for metabolite features by sex.
a) Model overview of the OPLS-DA model for the provided dataset, showing the R^2X, R^2Y and Q^2 coefficients for the groups (Male and Female). b) Permutation analysis, showing the observed (pink) and cross-validated (blue) R^2Y and Q^2 coefficients.

Table 0.3: Output from OPLSDA sex model S-plot along with corresponding VIP values.

Metabolite	p[1]	p(corr)[1]	VIP
Creatinine	0.27549	0.83449	2.8572
Citrulline	0.28376	0.74286	3.0786
Sarcosine	0.2158	0.58758	1.6958
GABA	0.24761	0.51792	2.4472
cis-Aconitic acid	0.24515	0.48237	2.724
β-Alanine	0.12627	0.45482	0.70673
Citric acid	0.1852	0.44703	0.84383
Ribulose-5-phosphate	0.12263	0.44449	0.82369
Isocitric acid	0.21912	0.39771	2.1385
Inosine	0.12335	0.39247	0.78589
Gluconic acid	0.091583	0.38912	0.14954
Glyoxylic acid	0.091254	0.38088	0.53769
Hydroxyproline	0.12839	0.37893	0.59623
Malic acid	0.089404	0.37613	0.32553
Guanosine monophosphate (GMP)	0.18069	0.36823	1.185
Lactic acid	0.15143	0.3672	0.3541
Tyrosine	0.10023	0.3661	0.035139
Threonine	0.10289	0.36533	0.0040469
Spermidine	0.078093	0.35967	0.34483
3-Phosphoglyceric acid	0.106	0.35216	0.96721
Alanine	0.098844	0.34014	0.15541
Valine	0.090363	0.33706	0.039038
Ornithine	0.10801	0.32348	0.20481
Methionine	0.10469	0.31104	0.55285
Histidine	0.076237	0.30659	0.23536
2-Oxoisovaleric-acid	0.20386	0.30528	2.6467
Glycine	0.080119	0.29608	0.020284
N.N-Dimethylglycine	0.10961	0.29401	0.75786
Glucose-1-phosphate	0.080837	0.29223	1.0281
Creatine	0.085623	0.28529	0.29888
Proline	0.083795	0.27386	0.10053
Inosine monophosphate (IMP)	0.10985	0.27044	0.88282
Pyruvic acid	0.11333	0.25751	0.9147
Glutamine	0.10691	0.25725	0.64903
Tryptophan	0.082756	0.24162	0.22317
Leucine	0.060968	0.24067	0.16795
Ethanolamine phosphate	0.096447	0.22581	0.44797
Glutamic acid	0.077948	0.22498	0.11667
Carnosine	0.077743	0.22476	0.25821
Adenosine monophosphate (AMP)	0.10243	0.21539	0.84452
2-Phosphoglyceric acid	0.049194	0.2145	0.55225
Lysine	0.067508	0.21384	0.50823

Metabolite	p[1]	p(corr)[1]	VIP
Glucose-6-phosphate	0.11745	0.2108	0.99899
Fumaric-acid	0.058516	0.1974	0.84955
Serine	0.058128	0.1968	0.13128
Betaine	0.071743	0.18678	0.49792
Uracil	0.038722	0.18235	0.34907
Isoleucine	0.050402	0.15271	0.38322
Choline	0.040908	0.15246	0.13937
Aspartic acid	0.034616	0.12663	0.42621
Homoserine	0.050824	0.11895	0.29098
Uridine diphosphate (UDP)	0.024667	0.11412	0.38436
Uridine	0.041775	0.10853	0.29927
2-Hydroxybutyric acid	0.031232	0.10034	0.221
Phenylalanine	0.029308	0.093128	0.469
3-Hydroxybutyric.acid	0.029264	0.080126	0.65323
Adenosine diphosphate (ADP)	0.032083	0.078068	0.077878
Guanine	0.015178	0.070223	0.38436
Succinic acid	0.021835	0.063789	0.37276
Arginine	0.0083901	0.019567	0.70061
2-Oxoglutaric acid	0.0085815	0.015868	0.45414
Hypoxanthine	0.0035884	0.0089486	0.59623
Guanosine diphosphate (GDP)	-0.0044877	-0.012625	0.0081925
Asparagine	-0.048699	-0.10995	1.407

Table shows metabolites ranked in order of decreasing correlation or reliability coefficient [p(corr)]. p[1], covariance or contributions; p(corr)[1], correlation or reliability coefficient; VIP, variable importance in the projection.

Table 0.4: Important metabolites identified by fold change analysis by infection status.

Metabolite	Fold Change (FC)	$\log_2$ (FC)
Choline	8.8175	3.1404
Lactic acid	6.1847	2.6287
Glutamic acid	2.4991	1.3214
Serine	2.4807	1.3107
Succinic acid	2.4599	1.2986
Histidine	2.453	1.2946
Phenylalanine	2.3656	1.2422
Lysine	2.3145	1.2107
Pyruvic acid	2.2875	1.1937
Hypoxanthine	2.1134	1.0796
Ornithine	2.1105	1.0776
Leucine	2.1069	1.0752
Homoserine	2.0195	1.014
Glucose-6-phosphate	0.48149	-1.0544
Spermidine	0.4527	-1.1434
Asparagine	0.44836	-1.1573
2-Hydroxybutyric acid	0.44462	-1.1694
Inosine monophosphate (IMP)	0.42322	-1.2405
Ethanolamine phosphate	0.41315	-1.2752
Guanosine monophosphate (GMP)	0.32008	-1.6435
Adenosine monophosphate (AMP)	0.25	-2.000
Adenosine diphosphate (ADP)	0.1844	-2.4391
Sarcosine	0.17928	-2.4797
Inosine	0.13699	-2.8679
3-Phosphoglyceric acid	0.1361	-2.8773

Table shows metabolites ranked in order of decreasing absolute fold change. Analysis is according to uninfected/infected ratio.

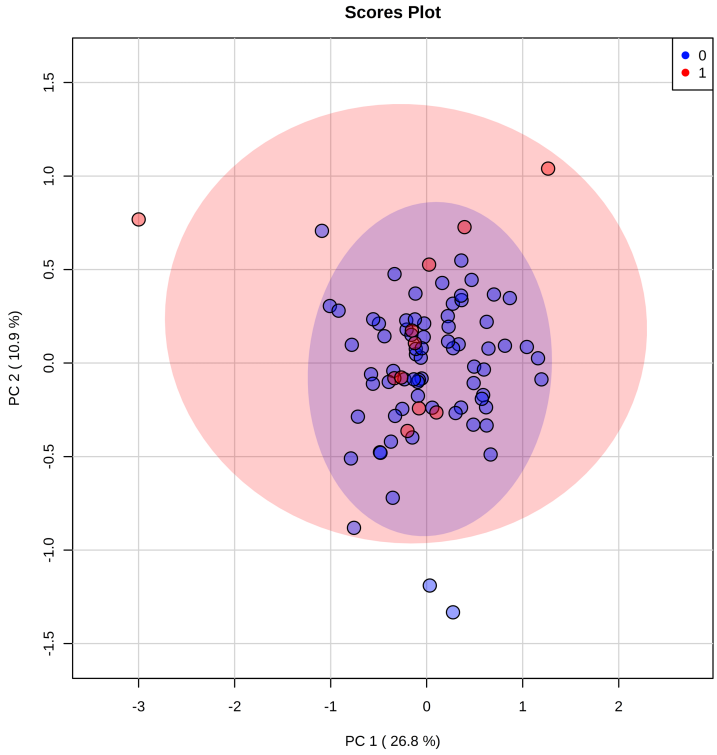
Table 0.5: Correlation pattern analysis of compounds showing patterns from negative to positive for infection.

Metabolite	Correlation	t-statistic	p-value	FDR
Adenosine diphosphate (ADP)	0.43076	4.2959	4.80E-05	0.002689
3-Phosphoglyceric acid	0.4095	4.0397	0.00012098	0.0033875
Adenosine monophosphate (AMP)	0.36253	3.5009	0.00075695	0.01413
Inosine	0.27435	2.5677	0.012077	0.11995
Asparagine	0.26342	2.4575	0.016124	0.11995
Lactic acid	-0.26245	-2.4479	0.016532	0.11995
Choline	-0.26204	-2.4438	0.016708	0.11995
Serine	-0.2554	-2.3774	0.019791	0.11995
2-Hydroxybutyric acid	0.25256	2.3492	0.021249	0.11995
cis-Aconitic acid	-0.25007	-2.3245	0.022602	0.11995
Sarcosine	0.24839	2.3078	0.023561	0.11995
Histidine	-0.23692	-2.1948	0.031042	0.13583
Guanosine monophosphate (GMP)	0.23626	2.1882	0.031533	0.13583
Glucose-6-phosphate	0.22272	2.0561	0.042991	0.15628
Ethanolamine phosphate	0.22243	2.0533	0.04327	0.15628
Glutamic acid	-0.22102	-2.0396	0.044652	0.15628
Creatinine	-0.2157	-1.9881	0.050184	0.1587
Succinic acid	-0.21244	-1.9566	0.053837	0.1587
Alanine	-0.21244	-1.9566	0.053844	0.1587
Glycine	-0.19987	-1.8358	0.070053	0.19615
Lysine	-0.19299	-1.7702	0.080451	0.21213
Leucine	-0.19122	-1.7533	0.083335	0.21213
Phenylalanine	-0.18497	-1.694	0.094112	0.22914
Aspartic acid	-0.17888	-1.6363	0.10566	0.24654
Ornithine	-0.17441	-1.5941	0.11481	0.25718
Threonine	-0.17199	-1.5713	0.12001	0.25848
Tyrosine	-0.13279	-1.2058	0.23141	0.46351
Inosine monophosphate (IMP)	0.13183	1.1969	0.23484	0.46351
Tryptophan	0.12799	1.1614	0.24888	0.46351
2-Oxoisovaleric acid	0.12791	1.1607	0.24917	0.46351
Spermidine	0.12594	1.1426	0.25658	0.46351
Pyruvic acid	-0.1144	-1.0364	0.30308	0.53039
Creatine	0.10995	0.99558	0.32242	0.53498
Citrulline	-0.10941	-0.99064	0.32481	0.53498
2-Oxoglutaric acid	-0.087052	-0.78646	0.43389	0.69423
Hypoxanthine	-0.084189	-0.7604	0.44922	0.69879
Isoleucine	-0.075729	-0.68353	0.49623	0.75104
Gamma aminobutyric acid (GABA)	-0.072465	-0.65391	0.51503	0.75406
Carnosine	0.069942	0.63102	0.5298	0.75406

Metabolite	Correlation	t-statistic	p-value	FDR
Homoserine	-0.068454	-0.61753	0.53862	0.75406
Hydroxyproline	0.060526	0.54574	0.58675	0.78979
Glutamine	0.059074	0.5326	0.59577	0.78979
Valine	-0.057369	-0.51717	0.60645	0.78979
3-Hydroxybutyric acid	0.052369	0.47197	0.63822	0.81228
Gluconic acid	-0.042401	-0.38196	0.70349	0.86895
Malic acid	-0.040099	-0.36118	0.7189	0.86895
Uridine	-0.038359	-0.34548	0.73063	0.86895
Ribulose-5-phosphate	0.033535	0.30198	0.76344	0.86895
Proline	-0.033532	-0.30196	0.76345	0.86895
Arginine	-0.031727	-0.28568	0.77585	0.86895
Methionine	-0.029174	-0.26267	0.79347	0.87126
Citric acid	0.026801	0.2413	0.80993	0.87223
N.N-Dimethylglycine	-0.023034	-0.20736	0.83625	0.88358
Isocitric acid	0.015931	0.1434	0.88633	0.91916
Betaine	-0.0096291	-0.086666	0.93115	0.93889
β-Alanine	0.0085451	0.076909	0.93889	0.93889

Table shows metabolites ranked in order of decreasing significance based on absolute FDR values. FDR, adjusted p-value (False discovery rate corrected).

a)



b)

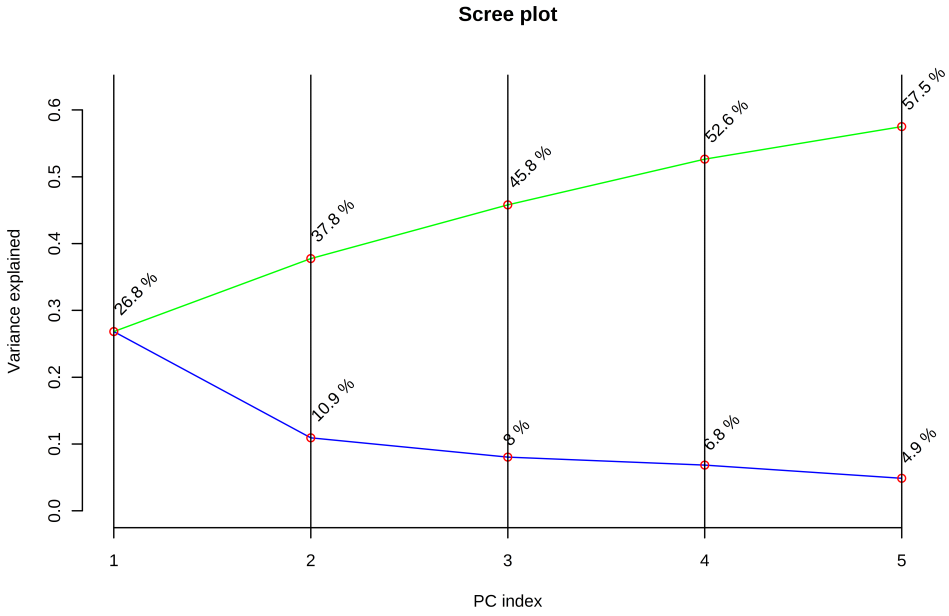
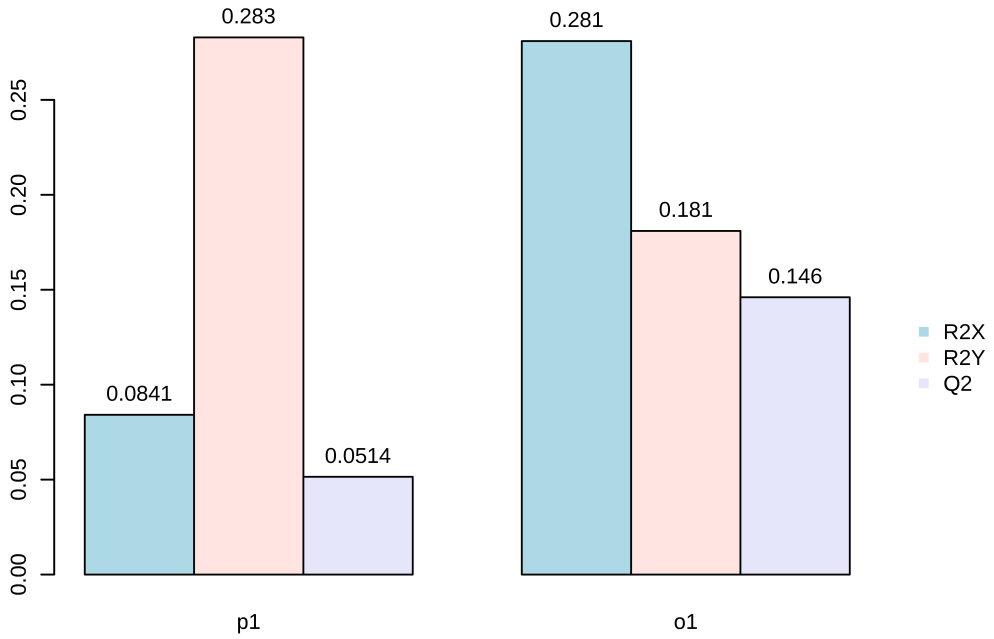


Figure 0.3: Principal component analysis (PCA) of metabolite features by infection status.
a) Scores plot between the selected principal components. The explained variances are shown in brackets. b) Scree plot shows the variance explained by principal components. The green line on top shows the accumulated variance explained; the blue line underneath shows the variance explained by individual principal components.

a)



b)

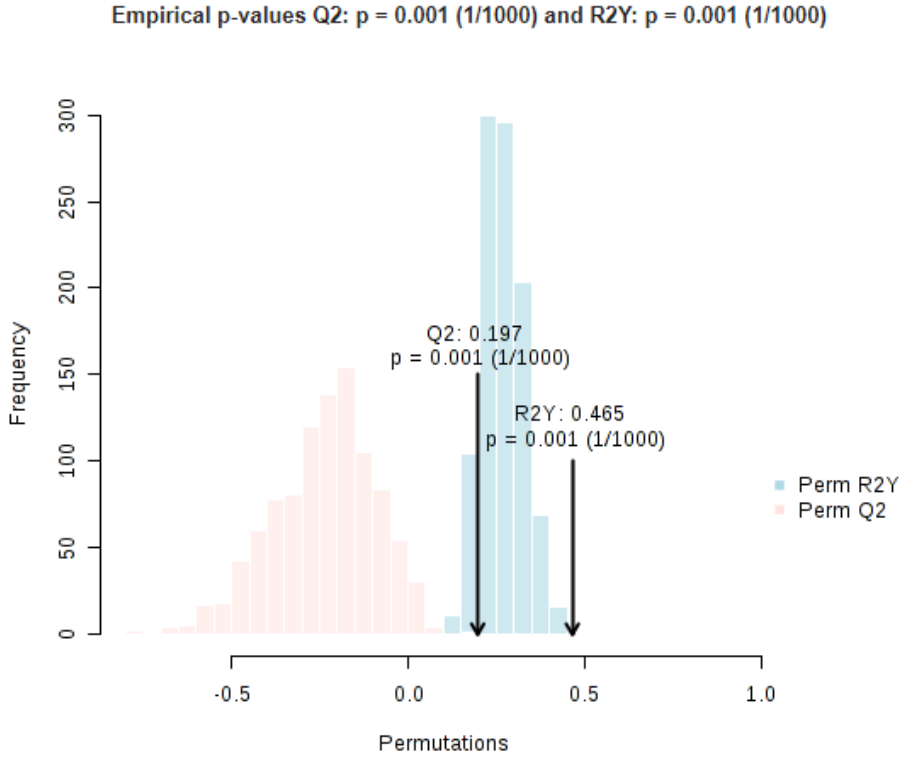


Figure 0.4: Orthogonal Projections to Latent Structures Discriminant Analysis (OPLSDA) model statistics for metabolite features by infection status.
a) Model overview of the OPLS-DA model for the provided dataset, showing the R^2X, R^2Y and Q^2 coefficients for the groups (schistosome negative and schistosome positive). b) Permutation analysis showing the observed (pink) and cross-validated (blue) R^2Y and Q^2 coefficients.

Table 0.6: Output from OPLSDA infection model S-plot along with corresponding VIP values.

Metabolite	p[1]	p(corr)[1]	VIP
Adenosine monophosphate (AMP)	0.16332	0.71903	1.5507
3-Phosphoglyceric acid	0.13835	0.71135	1.4998
Adenosine diphosphate (ADP)	0.17779	0.62789	2.297
Ethanolamine phosphate	0.19096	0.5937	1.3473
Glucose 6 phosphate	0.2032	0.55796	1.5275
Guanosine monophosphate (GMP)	0.16846	0.51693	1.4499
Inosine monophosphate (IMP)	0.11625	0.3841	0.75132
Asparagine	0.13774	0.32756	2.086
Sarcosine	0.06345	0.31531	0.94127
Carnosine	0.094955	0.31492	0.39715
Spermidine	0.053364	0.29941	0.4227
2-Hydroxybutyric acid	0.09136	0.27153	1.6003
Glutamine	0.10048	0.26156	0.42738
Inosine	0.056996	0.25414	1.1587
Creatine	0.079849	0.24129	0.68521
Ribulose-5-phosphate	0.048322	0.20786	0.14681
2-Oxoisovaleric acid	0.074067	0.17949	0.99399
Isocitric acid	0.068813	0.17527	0.11779
Hydroxyproline	0.043562	0.14883	0.33362
Tryptophan	0.058044	0.14599	0.95828
Citric acid	0.053018	0.12601	0.21236
β-Alanine	0.026035	0.10311	0.040634
N.N-Dimethylglycine	0.025649	0.093055	0.11956
Proline	0.01185	0.036949	0.20252
Betaine	0.011335	0.031553	0.065143
3-Hydroxybutyric acid	0.0062571	0.024438	0.25251
Pyruvic acid	0.0044649	0.014935	0.64409
Malic acid	-0.0055014	-0.025446	0.16326
Citrulline	-0.0087832	-0.026973	0.67094
cis-Aconitic acid	-0.010699	-0.028551	1.7647
gamma-aminobutyric acid (GABA)	-0.022744	-0.05116	0.60668
Methionine	-0.018806	-0.05124	0.20164
Gluconic acid	-0.017143	-0.071042	0.19269
Uridine	-0.026073	-0.079352	0.23735
Isoleucine	-0.030496	-0.090193	0.4822
Homoserine	-0.02843	-0.10138	0.36151
Arginine	-0.037684	-0.11043	0.20389
Valine	-0.035022	-0.12602	0.30024
Lactic acid	-0.044449	-0.12925	1.6997
Hypoxanthine	-0.052029	-0.12984	0.63531

Metabolite	p[1]	p(corr)[1]	VIP
Tyrosine	-0.049312	-0.17242	0.7152
2-Oxoglutaric acid	-0.049913	-0.18307	0.44697
Succinic acid	-0.066637	-0.18646	1.4298
Asp	-0.046762	-0.20107	0.78341
Creatinine	-0.046943	-0.20107	0.94836
Threonine	-0.069166	-0.24389	0.91853
Ornithine	-0.08025	-0.25436	1.0362
Glycine	-0.06732	-0.25854	0.98005
Alanine	-0.070685	-0.26489	1.0675
Phenylalanine	-0.080367	-0.29783	0.93998
Leucine	-0.085806	-0.32052	0.964
Lysine	-0.09314	-0.32526	1.0408
Glutamic acid	-0.11037	-0.33346	1.3776
Histidine	-0.09129	-0.35142	1.159
Serine	-0.10134	-0.36012	1.3535
Choline	-0.12508	-0.40257	1.5333

Table shows metabolites ranked in order of decreasing correlation or reliability coefficient [p(corr)]. p[1], covariance or contributions; p(corr)[1], correlation or reliability coefficient; VIP, variable importance in the projection.

Table 0.7: Correlation pattern analysis of metabolites showing patterns from positive for infection to negative post-treatment.

Metabolite	Correlation	t-statistic	p-value	FDR
Glucose-6-phosphate	-0.48165	-1.738	0.11285	0.24623
3-Phosphoglyceric acid	-0.47	-1.6839	0.12312	0.24623
Adenosine diphosphate (ADP)	-0.39272	-1.3504	0.20666	0.27554
Adenosine monophosphate (AMP)	-0.25002	-0.81657	0.4332	0.4332

Table shows metabolites ranked in order of decreasing significance based on absolute FDR values. FDR, adjusted p-value (False discovery rate corrected).

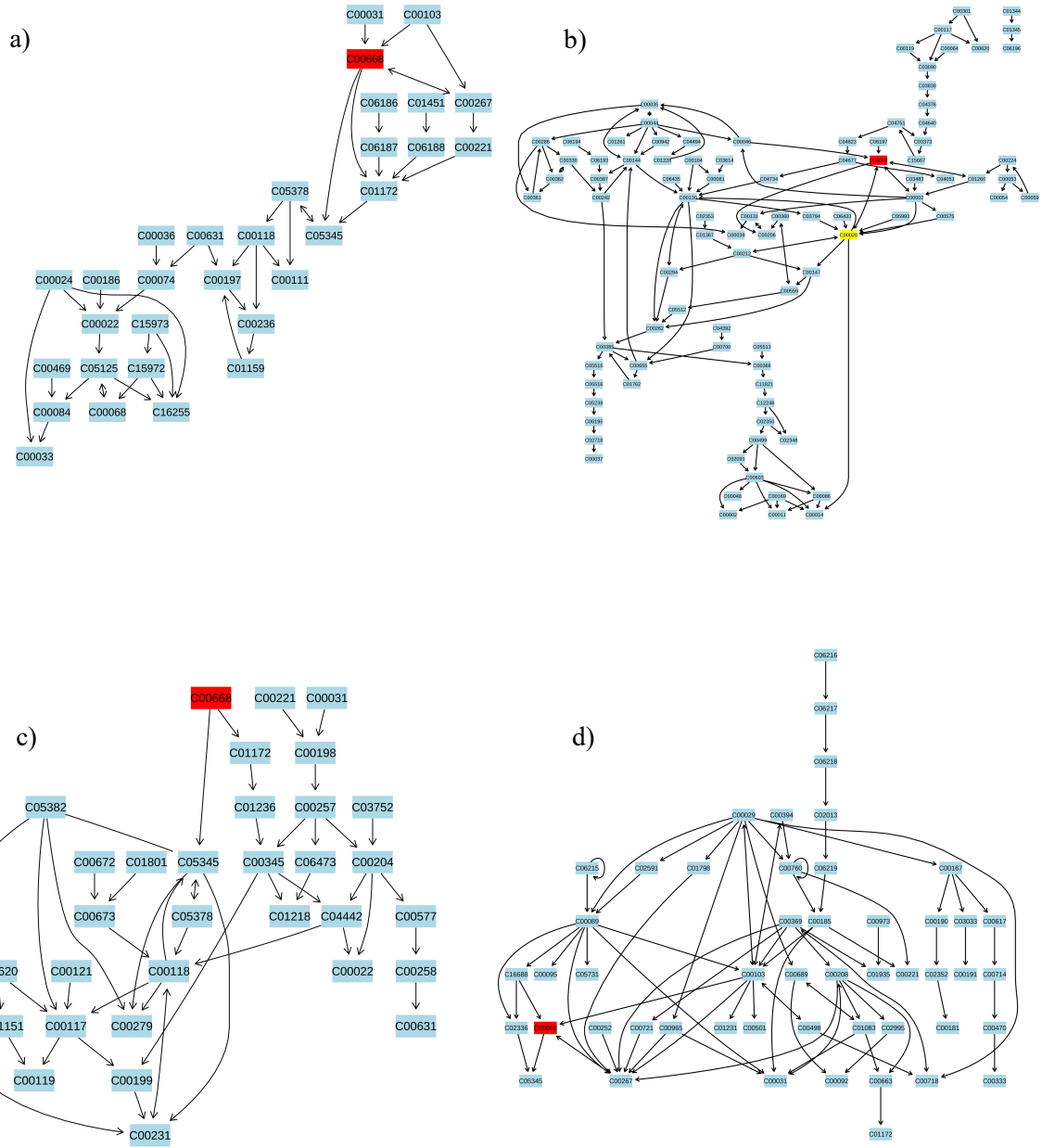


Figure 0.5: Detailed metabolic pathways of discriminatory metabolites for schistosome infection. a) Glycolysis/Gluconeogenesis (hits= glucose-6-phosphate). b) Purine metabolism (hits= AMP, ADP). c) Pentose phosphate pathway (hits= glucose-6-phosphate). d) Starch and sucrose metabolism (hits= glucose-6-phosphate). For compound colours within each metabolic pathway map–light blue are metabolites not in the data set used for pathway analysis and are used as background for enrichment analysis; other colours (varying from yellow to red) means the metabolites are in the data with different levels of significance. AMP, adenosine monophosphate; ADP, adenosine diphosphate

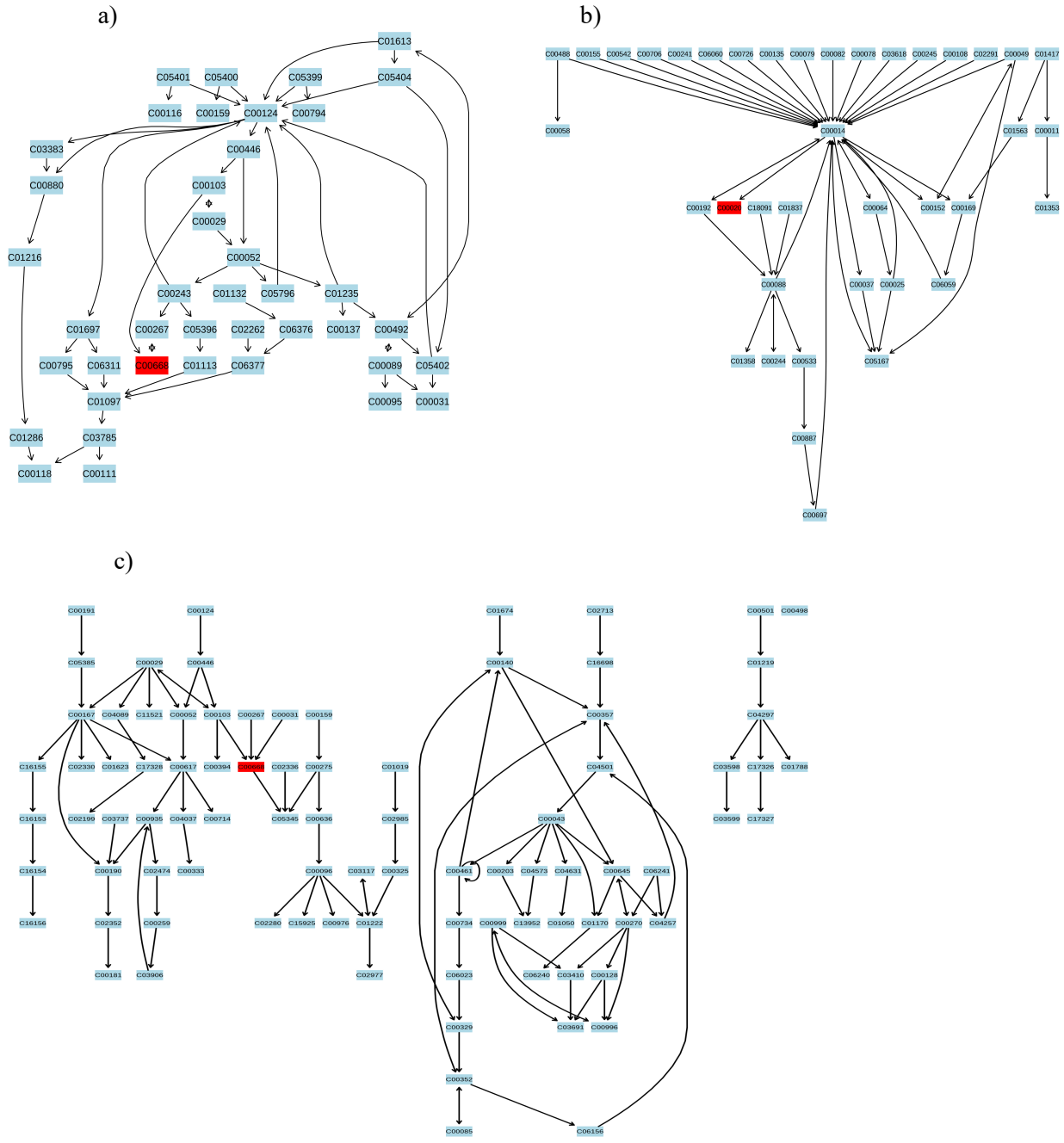


Figure 0.6: Detailed metabolic pathways of discriminatory metabolites for schistosome infection. a) Galactose metabolism (hits= glucose-6-phosphate). b) Nitrogen metabolism (hits= glucose-6-phosphate). c) Amino, sugar and nucleotide metabolism (hits= glucose-6-phosphate). For compound colours within each metabolic pathway map–light blue are metabolites not in the data set used for pathway analysis and are used as background for enrichment analysis; other colours (varying from yellow to red) means the metabolites are in the data with different levels of significance.

Table 0.8: Analysis output from pathway analysis.

Metabolic pathway	Total Cmpds	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Purine metabolism	92	2	3.61E-05	10.229	0.000253	0.000253	0.06428
Nitrogen metabolism	39	1	0.000757	7.1862	0.004542	0.002650	0
Glycolysis or Gluconeogenesis	31	1	0.042991	3.1468	0.21496	0.042991	0.06977
Pentose phosphate pathway	32	1	0.042991	3.1468	0.21496	0.042991	0.04348
Starch and sucrose metabolism	50	1	0.042991	3.1468	0.21496	0.042991	0.02439
Galactose metabolism	41	1	0.042991	3.1468	0.21496	0.042991	0.01724
Amino sugar and nucleotide sugar metabolism	88	1	0.042991	3.1468	0.21496	0.042991	0.00917

Table shows metabolites ranked in order of decreasing significance based on absolute FDR values. FDR, adjusted p-value (False discovery rate correction for multiple comparisons); Holm adjust, adjusted p value (Holm-Bonferroni correction for multiple comparisons); Raw p, unadjusted p values; Hits, number of metabolite hits from test data found in pathway, Total Cmpds, total number of compounds in pathway

Appendix C Supplementary information–Chapter 6

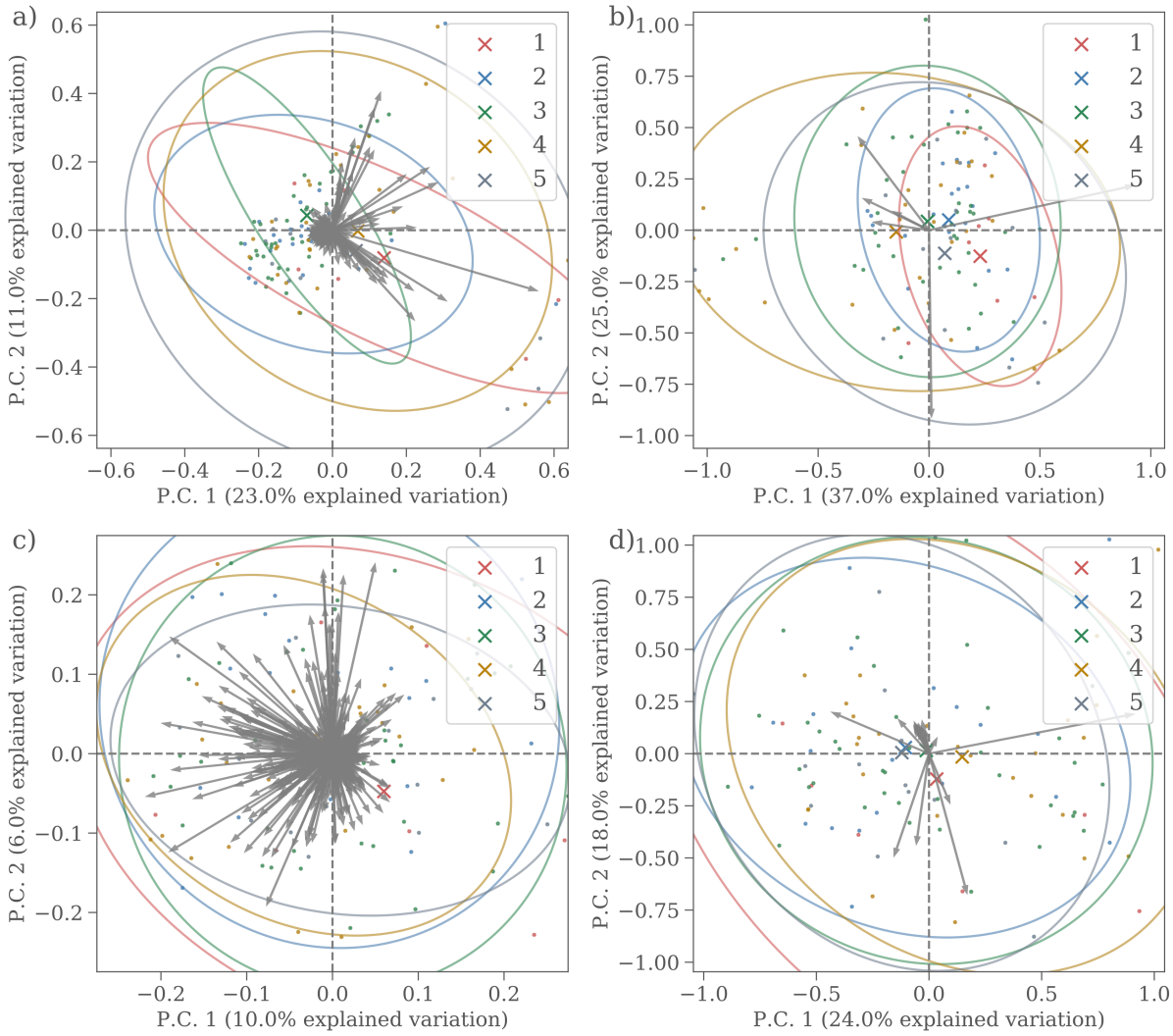


Figure 0.1: Principal component analysis (PCA) plots for microbiota across samples, annotated by age.
Figures a) and b) show PCA plots for fungi classified according to genus and phylum respectively. Figures c) and d) show PCA plots for bacteria classified according to genus and phylum respectively. Raw counts were clr-transformed, and the resulting matrices were ordinated using PCA. The projections of features are plotted as arrows and the projection of samples are plotted as points. The eigenvalues associated with the eigenvectors are used to describe the amount of explained variation per axis.

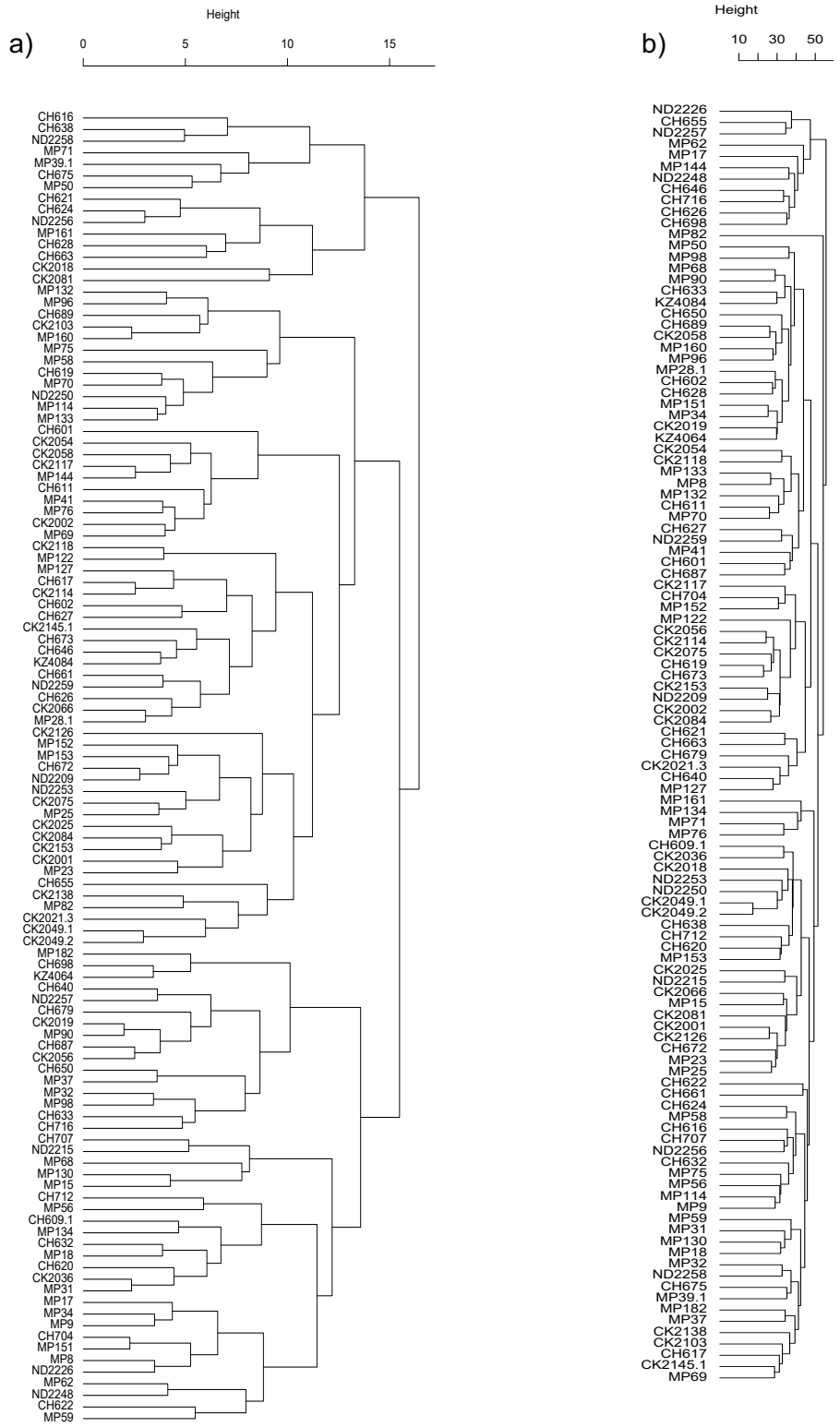


Figure 0.2: Bacteria abundance and composition dendrograms.
From read mapping to the genomic database, abundance was calculated for each microbial taxa across all samples. Clustered dendrograms show bacterial a) phyla and b) genera per sample. Raw counts were clr-transformed, the Aitchison distance (Euclidean distance) was calculated, and samples clustered based on distances (Complete-linkage-clustering).

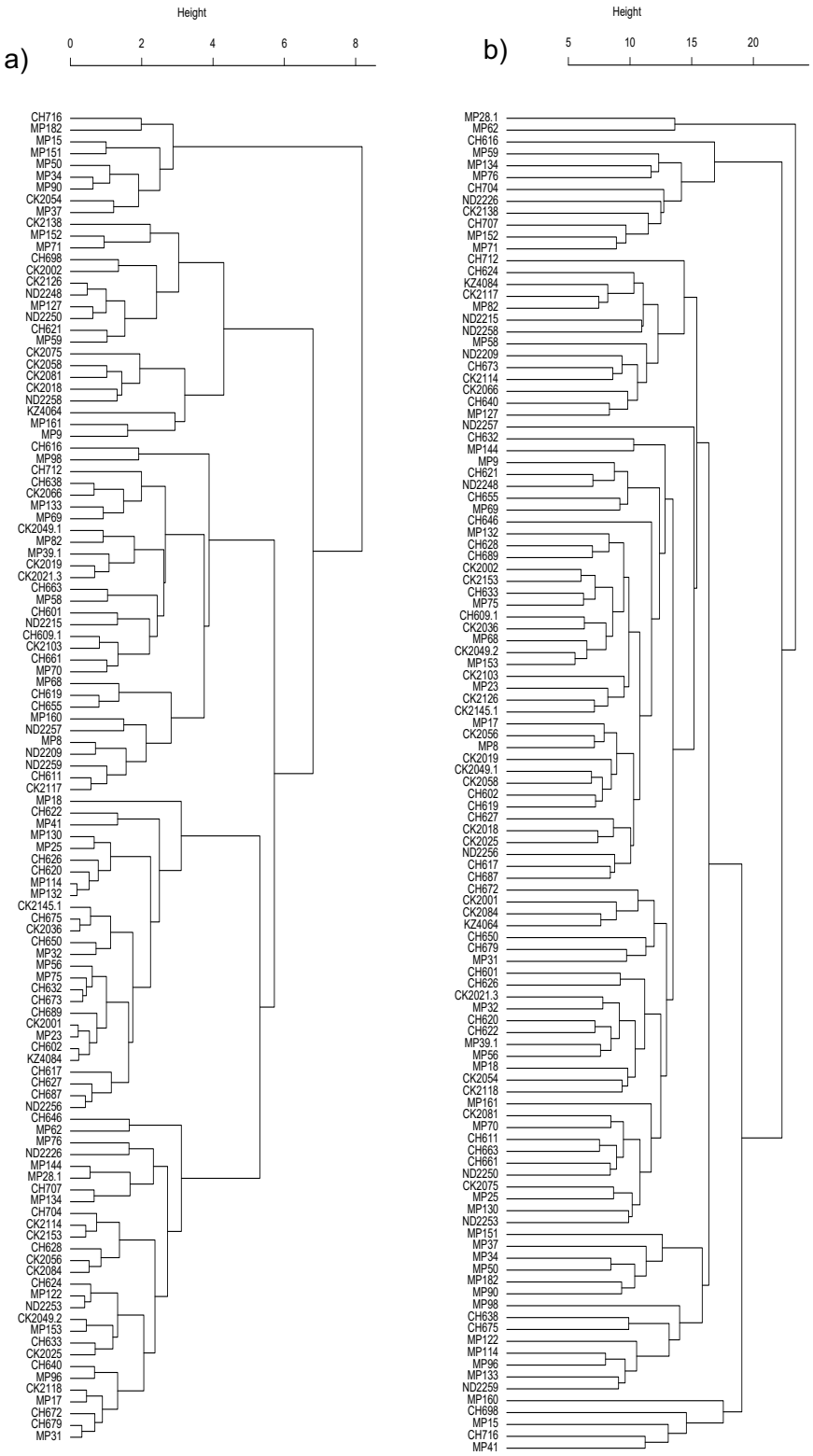


Figure 0.3: Fungi abundance and composition dendrograms.
From read mapping to the genomic database, abundance was calculated for each microbial taxa across all samples. Clustered dendrograms show fungal a) phyla and b) genera per sample. Raw counts were clr-transformed, the Aitchison distance (Euclidean distance) was calculated, and samples clustered based on distances (Complete-linkage-clustering).

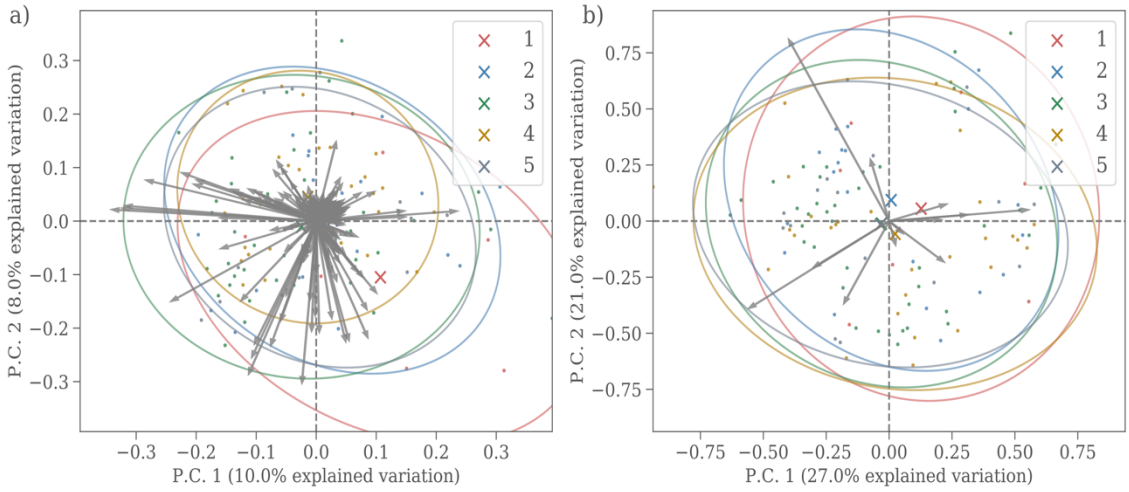


Figure 0.4: Principal component analysis (PCA) plots for AMR genes and drug classes across samples, annotated by age.
Figures a) and b) show PCA plots for AMR genes and AMR gene function classes respectively. Raw counts were clr-transformed, and the resulting matrices were ordinated using PCA. The projections of features are plotted as arrows and the projection of samples are plotted as points. The eigenvalues associated with the eigenvectors are used to describe the amount of explained variation per axis.

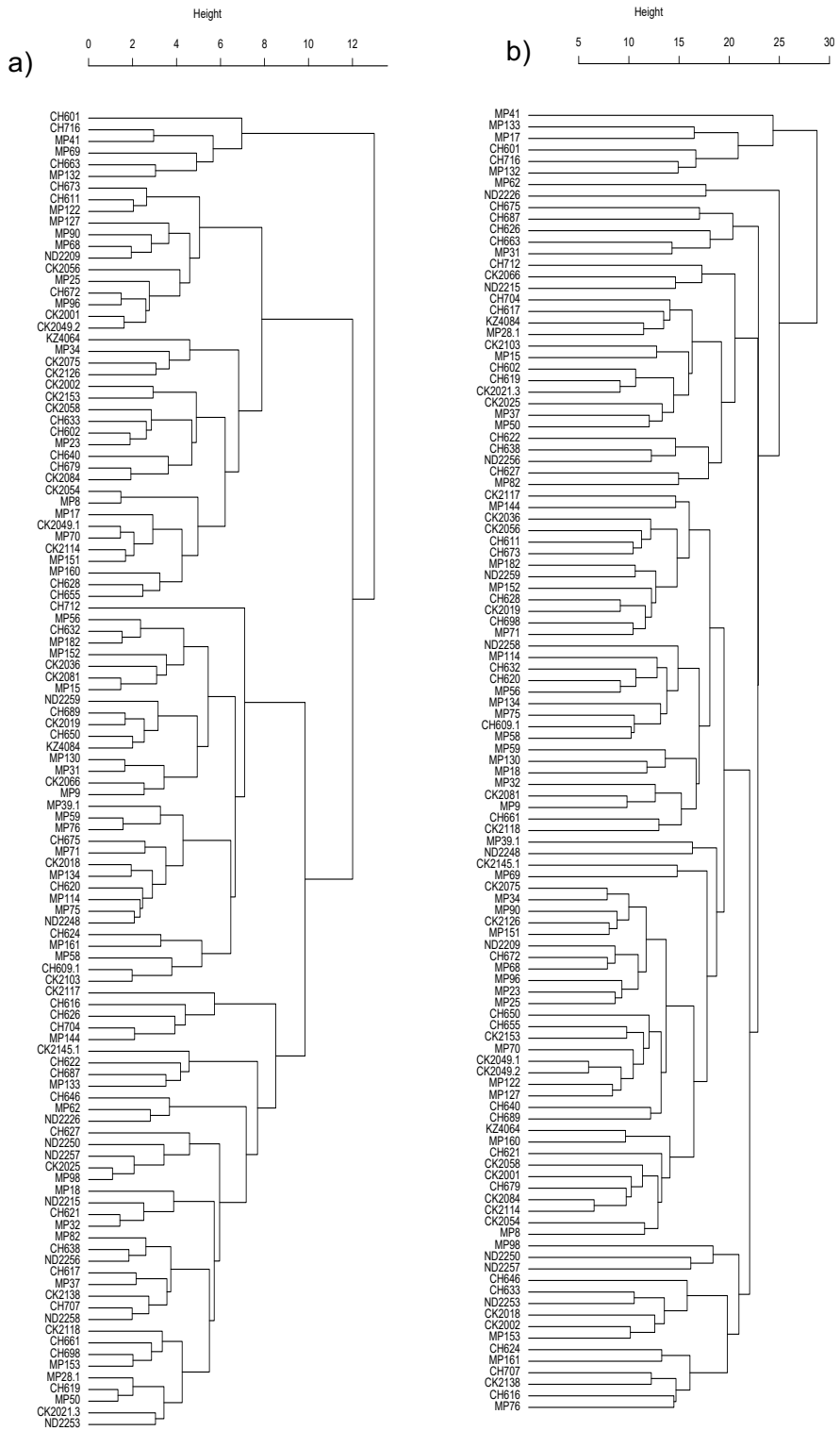


Figure 0.5: Antimicrobial resistance (AMR) genes and drug class dendrograms.
From read mapping to the ResFinder database, AMR abundance was calculated for each reference gene across all samples. Clustered dendrograms show AMR a) drug class and b) genes per sample. Raw counts were alr-transformed, the Aitchison distance (Euclidean distance) was calculated, and samples clustered based on distance (Complete-linkage-clustering).

Appendix D Survey questionnaire

Questionnaire
Pediatric Schistosomiasis/Bilharzia

This questionnaire is 3 pages long- please complete ALL pages.

Questionnaire to be completed at the time of visit with parents and enrollment of children into the study and only after obtaining parents consent and assent of participating child.

Date of questionnaire_____
Person administering questionnaire_____
Parent's full name_____

The following details/ questions are about the child.

Name_____ **Gender** M F ***(circle one)*** **Study ID #**_____

Date of birth (DD -MM -YY)_____ **Age (years)**_____

Years old <1 1-2 3-4 5-6 ***(circle one)***

Body weight _____kg **Height** _____cm

Village Name_____

QUESTIONS

1. **Has the child ever been treated for bilharzia or worms?**
   Yes No ***(circle either yes or no)***

   If yes where they treated _____

   Who treated them?_____

2. **Has the child ever been to hospital since birth (other than for vaccinations)?**
   Yes No ***(circle either yes or no)***

   If yes, what for and when? (You do not have to disclose any results of tests undertaken)

_____

1

3. Has the child received vaccination for:

a.	BCG vaccination (tuberculosis)?	Yes	No	Don't know	***(circle one)***
b.	Mumps	Yes	No	Don't know	***(circle one)***
c.	Measles	Yes	No	Don't know	***(circle one)***
d.	Rubella	Yes	No	Don't know	***(circle one)***
e.	Diptheria	Yes	No	Don't know	***(circle one)***
f.	***Polio***				
g.	***Hepatitis B***				
h.	others (specify) _____				

4. Please entre the Childhood vaccination Record from the Child health Card in the table below

Vaccine	Vaccine abbreviation	Dates of vaccination	Comments
BCG	BCG		
Polio	Polio		
Mumps	Measles		
Measles			
Rubella			
Diptheria, Pertusis, Tetanus	DPT		
Hepatitis B	HBV		

5. ***Has the child received a vitamin A supplement?***
Yes No ***(circle either yes or no)***

If yes when did they receive it? *(Put full date if available)*

_____

6. Domestic water source ***(circle as many as appropriate)***
a. unprotected well
b. River
c. Dam
d. Upgraded well
e. Borehole
f. Tap
g. Other (specify) _____

7. Where does the child normally go to the toilet? ***(circle as many as appropriate)***
a. Bush
b. cat sanitation
c. Latrine/toilet
d. Other (specify) _____

8. Is there a latrine at your home? ***(circle either yes or no***)
Yes No

9. If yes, are there any problems in using it? ***(circle either yes or no***)
Yes No
If yes, explain _____

10. Did your child do any of these activities in the water <u>yesterday</u>? *(circle as many as appropriate; if activity circled, ask other questions in table <u>for each separate contact</u>)*

	How many times?	Where? River/Dam/Well/ Other	(If River or Dam) <u>name</u> of place or nearest village/school	At what time? (e.g. 0800, 1600)
Swimming				
Playing in the water				
Bathing				
Laundry				
Washing dishes				
Washing (face and legs)				
Collecting water				
Fishing				
Crossing river				
Other_____				

11. How many times has your child been to the river or dam <u>in the last week</u>? _____

12. Where does the child normally get treated when unwell? (Circle as many as appropriate)

a. At home using herbal medicines
b. At home using western medicine
c. At the traditional healer
d. At the local clinic
e. Other please specify_____

ANY OTHER COMMENTS-
These can include any other comments the parent/guardian has about the child's health.

Appendix E Publications

- **Osakunor, D. N. M.**, M. E. J. Woolhouse and F. Mutapi (2018). "Paediatric schistosomiasis: What we know and what we need to know." PLoS Negl Trop Dis 12(2): e0006144.
- **Osakunor, D. N. M.**, T. Mduluza, N. Midzi, M. Chase-Topping, M. J. Mutsaka-Makuvaza, T. Chimponda, E. Eyoh, T. Mduluza, L. T. Pfavayi, W. M. Wami, S. A. Amanfo, J. Murray, C. Tshuma, M. E. J. Woolhouse and F. Mutapi (2018). "Dynamics of paediatric urogenital schistosome infection, morbidity and treatment: a longitudinal study among preschool children in Zimbabwe." BMJ Glob Health 3(2): e000661.
- **Osakunor, D. N. M.**, D. M. Sengeh and F. Mutapi (2018). "Coinfections and comorbidities in African health systems: At the interface of infectious and noninfectious diseases." PLoS Negl Trop Dis 12(9): e0006711.
- **Osakunor, D. N. M.**, D. M. Sengeh and F. Mutapi (2018). "Universal Health Coverage in Africa: Coinfections and Comorbidities." Trends Parasitol 34(10): 813-817.
- **Osakunor, D. N. M.**, P. Munk, T. Mduluza, T. N. Petersen, C. Brinch, A. Ivens, T. Chimponda, S. A. Amanfo, J. Murray, M. E. J. Woolhouse, F. M. Aarestrup and F. Mutapi (2020). "The gut microbiome but not the resistome is associated with urogenital schistosomiasis in preschool-aged children." Commun Biol 3(1): 155.
- Mduluza, T., C. Jones, **D. N. M. Osakunor**, R. Lim, J. K. Kuebel, I. Phiri, P. Manangazira, P. Tagwireyi and F. Mutapi (2020). "Six rounds of annual praziquantel treatment during a national helminth control program significantly reduced schistosome infection and morbidity levels in a cohort of schoolchildren in Zimbabwe." PLoS Negl Trop Dis 14(6): e0008388.

- Lim R. M, M. E.J. Woolhouse, T. Mduluza, M.Chase-Topping, **D. N.M. Osakunor**, L. Chitsulo, F. Mutapi. "Investigating a strategy for quantifying schistosome infection levels in preschool-aged children using prevalence data from school-aged children." PLoS Negl Trop Dis 14(10): e0008650.
- **Osakunor, D. N. M.**, T. Mduluza, D. Osei-Hyiaman, K. Burgess, M. E. J. Woolhouse, F. Mutapi. "*Schistosoma haematobium* infection is associated with alterations in energy and purine-related metabolism in preschool-aged children." PLoS Negl Trop Dis. *In Press.*

PLOS | NEGLECTED TROPICAL DISEASES

REVIEW

Paediatric schistosomiasis: What we know and what we need to know

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OPEN ACCESS

Citation: Osakunor DNM, Woolhouse MEJ, Mutapi F (2018) Paediatric schistosomiasis: What we know and what we need to know. PLoS Negl Trop Dis 12(2): e0006144. https://doi.org/10.1371/journal.pntd.0006144

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Abstract

Schistosomiasis affects over 200 million people worldwide, most of whom are children. Research and control strategies directed at preschool-aged children (PSAC), i.e., ≤5 years old, have lagged behind those in older children and adults. With the recent WHO revision of the schistosomiasis treatment guidelines to include PSAC, and the recognition of gaps in our current knowledge on the disease and its treatment in this age group, there is now a concerted effort to address these shortcomings. Global and national schistosome control strategies are yet to include PSAC in treatment schedules. Maximum impact of schistosome treatment programmes will be realised through effective treatment of PSAC. In this review, we (i) discuss the current knowledge on the dynamics and consequences of paediatric schistosomiasis and (ii) identify knowledge and policy gaps relevant to these areas and to the successful control of schistosome infection and disease in this age group. Herein, we highlight risk factors, immune mechanisms, pathology, and optimal timing for screening, diagnosis, and treatment of paediatric schistosomiasis. We also discuss the tools required for treating schistosomiasis in PSAC and strategies for accessing them for treatment.

Introduction

Schistosomiasis is a tropical and subtropical disease affecting communities with limited access to safe water and adequate sanitation provision [1–3]. It affects over 200 million people worldwide (90% in sub-Saharan Africa), of which a significant number (123 million) are children [3, 4]. The health impact includes poor growth and cognition in affected children [5, 6].

Despite the higher prevalence of schistosomiasis in children, preschool-aged children (PSAC), i.e., those aged ≤5 years, for a long time were considered to be at a low risk of infection [7]; and even if infected, the impact on their health was unknown or considered negligible. Operational difficulties, including obtaining parasitology samples for diagnosis, failure to detect light infections, and inadequate knowledge about risk factors in PSAC, have biased studies towards school-aged children (SAC), i.e., ≥6 years old and adults. Infection prevalence data from a number of epidemiological studies have led to the estimation that at least 50 million PSAC in Africa are infected with schistosomiasis [8], but the true global infection and

Competing interests: The authors have declared that no competing interests exist.

disease burden remains to be quantified. This makes it difficult to make operational and economic plans for controlling schistosomiasis in PSAC. Furthermore, gaps relating to infection, disease dynamics, and treatment need addressing if we are to deliver sustainable schistosome infection and disease control in PSAC and strengthen schistosomiasis elimination programmes.

Here, we summarise the current knowledge of paediatric schistosome infection, disease dynamics, and treatment. We also identify important knowledge gaps in paediatric schistosomiasis practice.

Epidemiology of paediatric schistosomiasis

In schistosome-endemic areas, a significant amount of the exposure to infection in PSAC is passive (i.e., use of contaminated water in the home or children being bathed/sitting in a dish of fresh water while the guardian conducts domestic chores), particularly in the youngest children. Exposure becomes more active as the children grow (e.g., accompanying caregivers to water sources for domestic chores) [9, 10]. Therefore, in the early years of infants and young children, exposure to infection is closely linked to that of the caregiver. This disassociates as children grow older, become independent, and frequently visit contaminated water sources with friends and/or older siblings.

Exposure to infection is incremental, and almost all children in high transmission areas will have been exposed to schistosome cercariae by age one [11], with infection prevalence and intensity increasing as children grow up [12]. Thus, there is a need for inclusion of PSAC in large-scale projects that map the distribution of schistosomiasis, to inform planning for drug procurement and operational strategies for including these children in national control programmes.

In addition to the lack of burden estimates of schistosome infection and disease in PSAC, there is a paucity of incidence data in this age group. Longitudinal studies tracking the incidence of schistosome infection are required to identify and quantify exposure patterns, risks, and health impacts of infection at an early age and, most importantly, to plan treatment strategies.

Risk factors for schistosome infection

Several factors influence the risk for schistosome infection in PSAC, including those already identified in other age groups [Fig 1]. Environmental factors (including temperature, seasonal rainfall patterns, and altitudes) influence the survival of the intermediate host snail, as well as parasite development in the snail, affecting the force of transmission and infection [13, 14]. Exposure patterns of the human host are also affected by climatic changes (e.g., hotter seasons prompt increased recreational use of infected water sources), and passive contact with infective water can increase amongst PSAC, while they are waiting for their caregivers to complete chores. Exposure will vary in both the surface area exposed as well as the duration and frequency of exposure [15].

In addition to these well-studied risk factors, we are beginning to appreciate the impact of the human gut and urinary microbiome [22–24]. The gut microbiome is believed to be particularly variable in the early years of life [25]; for example, we have reported that the relative abundance of different bacterial taxa varies within the first three years of life [26]. The gut microbiome plays a vital role in maintaining barrier integrity, and subsequently impacts the host immune system [27], and contributes to the observed differences in infection patterns [28]. Recently, we demonstrated a significant difference in the microbiome structure (frequency and diversity of the host bacteria species) between schistosome-infected and

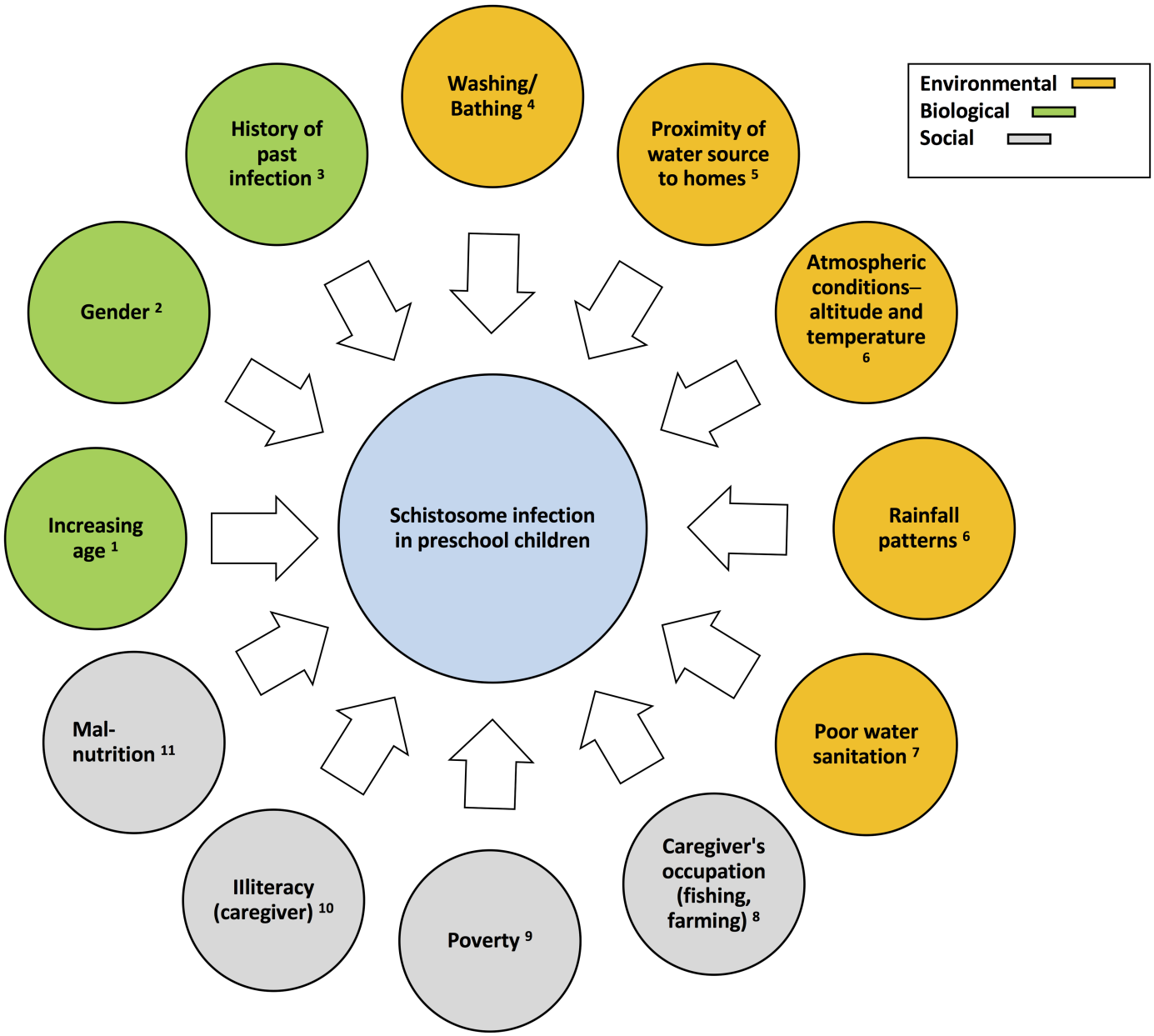

Fig 1. Risk factors for schistosome infection in PSAC. *Adapted from*: **1** [14]; **2** [14, 15]; **3** [16]; **4** [15], **5** [15, 17, 18]; **6** [13, 14]; 7 [19]; **8** [14]; **9** [18]; **10** [20]; **11** [21].

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uninfected Zimbabwean children aged 1 to 10 years [26]. From the study design, the mechanistic direction of this relationship and the influence by other factors was unclear. These aspects together with any mechanistic pathways are yet to be elucidated. In this age group, the impact of gut microbiome dysbiosis on nutritional status is of particular importance.

Emerging ideas from initiatives such as the Human Microbiome Consortium suggest that our focus on PSAC should be to understand how the microbiome functions and influences innate susceptibility to schistosome infection, if at all [29]. Helminths modulate host immune responses for their survival, and this in turn has a negative impact on the host microbiome

structure and nutrition [23]. Increasing research in nutraceuticals should thus inform additional child supplementation programmes in affected countries. At present, schistosomiasis interventions targeting the microbiome remain theoretical, as mechanistic studies to establish and quantify causal relationships are lacking.

In schistosome-endemic areas, malnutrition and undernourishment are significant childhood problems. Nonetheless, there are no studies detailing the attributable fraction of malnutrition due to schistosomiasis or more importantly, the impact of treatment on these factors. This information is necessary to advocate for control strategies to prioritise PSAC. Studies have also yet to establish the role/relevance of anthropometric measures as a tool for identification of the risk of malnourishment among PSAC in schistosome-endemic areas.

Schistosome-specific immune responses and clinical relevance

The interest in immunity in schistosome infections is twofold. First, hosts in endemic areas develop protective acquired immunity against infection/reinfection, and second, the severe and chronic clinical manifestations of schistosomiasis are immune mediated. Decades of studying immune responses in both human and experimental models have given insights into the aetiology and clinical manifestation of morbidity and immunopathology and into the development of schistosome-specific protective immune responses. The paradigms presented by these studies are not easy to extrapolate to PSAC, due to differences in the natural history of infection. Human studies have largely focused on SAC and adults with distinct histories of infection, which impacts on their immune phenotype [30]. Furthermore, most experimental studies on schistosome immunology use mouse models, which are limited in their ability to recapitulate morbidity and pathology occurring in the natural human host. For instance, eggs implanted in mice were recently used to develop an experimental model for urogenital schistosome pathology [31], but the relevance of this to the human disease forms in young children is unclear.

Acute and chronic stages of schistosome infection

In endemic areas, the acute stage of schistosome infection—including the Katayama syndrome—receives very little research or clinical intervention compared to other areas [32, 33]. As detailed above, the first infection event in most endemic areas occurs early at the pre-school age, but to date, no studies of schistosome cercarial dermatitis in PSAC have been published. The immune responses occurring in the early stages of infection are poorly documented in humans, and these events in PSAC would be very informative on the nature and development of both pathological and parasite-protective immunity.

In studies of SAC, protective immunity against schistosomiasis is characterized by a dynamic shift in the balance between effector and regulatory (humoral and cellular) immune responses, with effector responses surpassing the regulatory responses as infection progresses and exemplified by Nausch and colleagues [34]. We have previously proposed a threshold hypothesis [35] to explain the switch from a predominantly regulatory phenotype to an effector immune phenotype as infection progresses. Nonetheless, there is a paucity of immunology studies in PSAC detailing the dynamics occurring at the transition of infection from acute to chronic. Knowledge of these responses will be informative for schistosome vaccine development and deployment, as the ideal vaccine would target all children at risk of schistosome infection who may have already experienced their first schistosome infection exposure/infection event.

Drug-induced protective immunity

Studies in SAC and adults have shown that treatment of schistosome infections with Praziquantel (PZQ) enhances schistosome-specific immune responses by (i) removing the

immuno-suppressive effects of the adult worms [35–37], and (ii) introducing large amounts of parasite-specific antigens to the immune system to reach the threshold required to induce an immune response [38–40]. These changes are associated with reduced reinfection rates [38, 41]. One of the consequences of the previous schistosome treatment guidelines was that studies on the effects of PZQ were focused more on SAC and adults. Thus, the immunological consequences of PZQ in PSAC remain poorly characterised. In a recent study in Zimbabwean PSAC, we showed an increase in anti-parasite IgM and IgE titres, six weeks after treatment with PZQ, and this was associated with resistance to reinfection [42]. This mimics the development of naturally acquired immunity, albeit (in this case) accelerated by treatment [39, 40, 43].

Evidence from experimental studies suggest that a more rational approach to the timing of helminth treatment may have additional benefits beyond the transient removal of infection and may also speed up the development of protective immunity more effectively than treating chronic infection. These studies have shown that treatment of the first helminth infection induces earlier and greater levels of protection against reinfection by preferentially inducing the effector over regulatory responses [44–46]. In this case, chronic infection induces a constant state of anti-parasite Th2 response, reducing the overall effect of Th1 effector responses required to fight infection [47]. This suggests that treatment of the first schistosome infection could have a longer-lasting impact on susceptibility to reinfection and presents the possibility of targeting treatment for maximum benefit in terms of the future health of the child. As detailed above, a single PZQ treatment can induce resistance to reinfection in PSAC [48], suggesting that if treatment of PSAC is optimally timed then repetitive treatments may be reduced or not required [49, 50]. Longitudinal studies determining the optimal treatment time are needed to inform the treatment guidelines for PSAC. Now, we have a window to conduct these studies, while there is a concerted effort to make a paediatric formulation of PZQ for deployment within the next few years.

Pathology and morbidity

Schistosome pathology and morbidity are still being defined in older children and adults, e.g., only recently was urinary schistosomiasis renamed urogenital schistosomiasis to reflect the increased recognition of the genital manifestations of *S. haematobium*-related disease [51]. Pathology and morbidity remain poorly described and studied in PSAC [10] compared to SAC. However, studies are beginning to shed light on schistosome morbidity in this group, e.g., a recent study using ultrasound has contributed to describing urinary morbidity in PSAC [52].

Describing morbidity and pathology at all stages of schistosome infection in PSAC will inform overall healthcare in this age group. In PSAC, clinical symptoms upon schistosome exposure and infection (e.g., cercarial dermatitis and fever) may go unrecognised [5, 53, 54] or be mistaken for symptoms of other illnesses such as malaria, which present with similar symptoms (e.g., fever).

It is also possible that schistosome infections have wider impacts in childhood health. In PSAC, chronic prenatal exposure/sensitisation to helminth infection is reported to be associated with reduced efficacy of childhood vaccines through induction of a persistent Th2 response phenotype [55, 56]. Chronic exposure is also thought to be associated with environmental enteropathy, which affects the efficacy of vaccines at infancy [57]. Reduced vaccine efficacy means infection and disease from the vaccine-preventable infections. Thus, controlling schistosomiasis in PSAC may have the added health benefit of improving vaccine efficacy; appropriate studies are required to investigate if this would be the case.

Schistosome infections damage epithelial barriers, resulting in anaemia, poor nutrition, and growth [58, 59]. Thus, even with the low parasite burdens in PSAC, the pro-inflammatory response generated can quickly lead to chronic morbidity [60]. For example, schistosome-associated anaemia and malnutrition in older Kenyan children (5–18 years old) was attributed to pathology beginning much earlier in life [58]. The nutritional effects could also be attributed to microbiome dysbiosis as reported in the experimental mouse model of helminth infection [61]. Thus, the impact of schistosomiasis in PSAC should be an integral part of interventions, targeted at improving early child health and development.

Poor awareness, recognition, and lack of understanding and quantification of schistosome-associated morbidity has previously made schistosome control in PSAC a lower priority than in older children and adults, but concerted efforts are now beginning to correct this. There is a need for biomarkers of environmental enteropathy in PSAC [59], mechanistic studies to infer causality, and well-defined predictors of growth and nutrition in PSAC exposed to schistosomiasis.

Paediatric schistosomiasis in coinfected hosts

Similar to all other age groups in tropical regions, PSAC are at risk of coinfections and therefore, comorbidities. Studies in older children (5–18 years old) indicate that the presence of multiple infections in endemic areas lead to significant morbidity, including anaemia and malnutrition and these are important in the formative years of PSAC [58]. Specific coinfection studies include malaria–schistosomiasis, where studies in SAC suggest that malaria severity is compounded by schistosome infection, and malaria treatment is more effective when schistosome infection is also treated [62, 63]. To date, no detailed studies have been conducted on the fraction of malaria deaths in PSAC that are attributable to schistosome coinfection. In addition, *S. haematobium* infection has been shown to reduce the level of protective IgG responses to malaria vaccine candidates [64]. In another instance, in vitro studies have shown that *Salmonella* can evade optimal antibiotic treatment by binding to schistosomes using fimbral proteins (FimH) present on its surface [65], and that effective treatment of schistosomiasis results in the release of *Salmonella*, causing septicaemia [66]. These findings need validation in PSAC to allow the implementation of appropriate interventions.

Diagnosis of infection

One operational model being proposed for the treatment of schistosomiasis in PSAC is that diagnosis is made before treatment, unlike the mass drug administration (MDA) approach where SAC are treated without diagnosis [67]. This means that in the "diagnose and treat" model, accurate point of care (POC) diagnostics are key to guide the targeted treatment.

Direct parasitological methods (Kato–Katz and urine filtration) are recommended by the WHO [68] and are convenient, specific, rapid, cheap, and suitable for field applications in PSAC [69, 70]. Nonetheless, in addition to the already known limitations of low sensitivity of the parasitological methods in SAC and adults, these methods have the added operational challenge of obtaining adequate urine and stool samples in PSAC [71], particularly the collection of replicate samples on different days to improve sensitivity [72].

Various other methods have been developed in the laboratory to address the issues of sensitivity and are at different stages of translation into field tools. Immunological detection of schistosome-specific antibodies in sera has been shown to be more sensitive in diagnosing infection in PSAC than parasitological methods [12]. The non-invasive detection of worm antigens in urine, i.e., circulating anodic antigen (CAA) [73, 74] and circulating cathodic antigen (CCA) [75] have also been evaluated in PSAC. These have been shown to be a reliable tool

in diagnosing *S. mansoni* [76] but less so in *S. haematobium* diagnosis [77]. Issues of cost, which pose challenges in low resource settings where schistosomiasis is endemic [78, 79], would also need to be overcome. Molecular techniques detecting parasite DNA in urine have shown promise in PSAC [80, 81], although the absence of POC versions of these tests means they require expertise and specialised equipment [82]. Compared to CCA and parasitology, polymerase chain reaction (PCR) was most effective in detecting low intensity *S. mansoni* and *S. haematobium* infections [83, 84]. The use of cell-free parasite DNA present in urine and saliva [85] in PCR techniques can ease sampling difficulties in PSAC and detect early infections [86]. MicroRNAs have been characterised in animal models of *S. japonicum* [87] and *S. mansoni* [88]. Despite conflicting reports on the utility of miR–223 as a biomarker for helminth infections [88, 89], microRNAs have great diagnostic potential in PSAC and require further exploration. However reliable these diagnostics turn out to be at the lab bench, their true utility will be tested in the field.

Morbidity markers for schistosomiasis

The majority of morbidity biomarkers associated with schistosomiasis are non-specific, relating to physiological, biochemical, and immunological changes. As such, their interpretation in the light of coinfections and comorbidities is complex. Recent years have seen studies conducted in PSAC to evaluate the utility of morbidity markers described in SAC. These have included evaluation of the classic urine markers, haematuria and proteinuria in *S. haematobium* infection (e.g., in Nigeria [90] and Zimbabwe [91]), and the urine albumin-creatinine ratio (UACR) in Zimbabwe [91]. Faecal occult blood (FOB), the presence of cryptic blood in stool, results when *S. mansoni* eggs perforate the intestinal mucosa and cause a small release of blood into the bowel [92, 93], along with calprotectin released by granulocytes in response to the accompanying inflammation [94]. FOB and calprotectin have been evaluated in PSAC in Uganda [92, 95]. In these studies, FOB and calprotectin correlated positively with *S. mansoni* infection pre- and post-treatment although there may be exceptions.

The challenge with these non-specific markers particularly is to determine how schistosomiasis influences them; this is a difficult task because even determining the fraction attributable to schistosome infection does not indicate causation. Equally important is the lack of specific information on what they mean in terms of the child's overall current and future health. There is the need for a knowledge base addressing these two issues if these morbidity markers are to form part of the diagnostic tool kit to inform the "diagnose and treat" approach for PSAC.

Schistosome treatment in PSAC

Following the recommendation to treat PSAC infected with schistosomes, there is a need for a drug formulation that is suitable for PSAC. PZQ is the drug of choice for treating schistosomiasis at a recommended dose of 40–60 mg/kg body weight. In ill-resourced endemic areas, a height–dose pole has been developed as a surrogate to weight scales for operational purposes [96]. Modifications were made to extend the original dose pole used for SAC and adults to include PSAC [97, 98]. PZQ is currently administered to PSAC as crushed tablets with juice or bread [4, 10]. Followed by an endorsement from the WHO [99], a recent randomised dose-ranging trial reports that a single 40 mg/kg dose of PZQ can be used for treatment in PSAC [100]. Although PZQ is confirmed safe and efficacious [101], there is little information on its pharmacokinetics in PSAC [102, 103], and this is compounded by variability in bioavailability, influenced by brand [104, 105].

PSAC tolerate PZQ well with few reports of adverse effects: normally abdominal pain, vomiting, fatigue, and diarrhoea that resolve within 24 hours [98, 100, 101]. Contrary to suggestions of a higher efficacy dose (>40 mg/kg) in PSAC [106], a recent study reported on the efficacy and safety of escalating doses (20 mg/kg, 40 mg/kg, and 60 mg/kg) of PZQ in PSAC and SAC [100]. PZQ showed a flat dose-response curve in PSAC compared to that in SAC and the current 40 mg/kg remains the best option in PSAC [99]. In PSAC, PZQ can be administered with other antihelminthics (e.g., Albendazole) as a deworming package. There are currently no drug–drug interaction studies that have been conducted on these drugs in PSAC [107]. PZQ is metabolised by the cytochrome P_{450} enzymes as shown in experimental studies [108, 109]. With growing evidence of genetic diversity for cytochrome P_{450} variants in schistosome-endemic regions in Africa [110], there are implications for PZQ metabolism and treatment efficacy which remain to be elucidated in PSAC.

Paediatric PZQ formulation

Operationally, the large size and bitter taste of the existing tablet are associated with administration difficulties in PSAC [100, 111]. To address these issues, the Paediatric Praziquantel Consortium has produced a paediatric PZQ tablet meeting a previously suggested target product profile (TPP) [4, 112]. This formulation—a smaller, orally dispersible tablet with a masked taste—has successfully undergone phase I clinical trials and phase II trials are currently underway (http://www.pediatricpraziquantelconsortium.org/).

Accessing PSAC for schistosome treatment and control

Preventive chemotherapy programmes targeting SAC are conducted via MDA with antihelminthics (usually PZQ and Albendazole) administered in the school setting. PSAC do not go to school (although some may be in early child development centres) and the "diagnose and treat" recommendation makes it difficult to treat them outside a health setting. One strategy is to access PSAC through the Expanded Programme on Immunization (EPI) in primary health centres [4], and our studies in Zimbabwe confirm the feasibility of accessing PSAC at health centres [101]. However, in some health systems, the EPIs may already be "crowded," implementing other interventions at the same time; hence, alternative access strategies are required. A report from a recent meeting [67] included the approach of empowering health workers to identify clinical cases (i.e., non-specific signs of suspected cases) of schistosomiasis in PSAC and treating them, as a potential means of accessing PSAC. This approach has both advantages and disadvantages; the main disadvantage is the potential of treating non-specific clinical symptoms due to infections/diseases other than schistosomiasis.

Conclusion

There is now a global move to elevate research on paediatric schistosomiasis and to promote control, not only for child health but also to move towards eliminating schistosomiasis. The control of paediatric schistosomiasis will only be prioritised in countries with limited health budgets when (i) there is compelling evidence on the burden of infection and disease on child health and development, (ii) there are cost-effective intervention tools, and (iii) engagement at stakeholder and end-user levels. The research community can contribute to all three aspects, and bridging the knowledge gaps highlighted in this review will make a significant contribution to the control of schistosomiasis in PSAC.

Key learning points

- Preschool-aged children (PSAC) carry significant infection and morbidity to schistosomiasis. There is a treatment gap in mass drug administration, and studies mapping the level of infection and morbidity in this group. Infection and disease quantification is critical to inform planning and deployment of schistosome treatment in PSAC.
- Existing and new morbidity markers, along with improved multiple diagnostic tools, are useful for targeted disease identification and treatment in PSAC. The utility of these along with a proper strategy is important. There is a need for better-defined disease markers for this age group.
- Treatment with Praziquantel is safe and efficacious. Owing to existing challenges with the current formulation, there is a need for a paediatric formulation, a better dosing system, a better treatment strategy, and innovative ways of accessing PSAC for treatment.
- Most PSAC will experience infection early, and there is a need to characterise and quantify the consequences of these early infections in terms of impact on the overall health of children.
- PSAC are capable of mounting an adaptive immune response to schistosome infections, and a single treatment can illicit this response. Exploring the applicability of this in resistance to reinfection and morbidity is important.

Top Papers

- Wami WM, Nausch N, Midzi N, Gwisai R, Mduluza T, Woolhouse M, et al. Identifying and evaluating field indicators of urogenital schistosomiasis-related morbidity in preschool-aged children. PLoS Negl Trop Dis. 2015;9(3):e0003649. doi: 10.1371/journal.pntd.0003649. PubMed PMID: 25793584; PubMed Central PMCID: PMCPMC4368198
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Dynamics of paediatric urogenital schistosome infection, morbidity and treatment: a longitudinal study among preschool children in Zimbabwe

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ABSTRACT

Background Recent research has shown that in schistosome-endemic areas preschool-aged children (PSAC), that is, ≤5 years, are at risk of infection. However, there exists a knowledge gap on the dynamics of infection and morbidity in this age group. In this study, we determined the incidence and dynamics of the first urogenital schistosome infections, morbidity and treatment in PSAC.

Methods Children (6 months to 5 years) were recruited and followed up for 12 months. Baseline demographics, anthropometric and parasitology data were collected from 1502 children. Urinary morbidity was assessed by haematuria and growth-related morbidity was assessed using standard WHO anthropometric indices. Children negative for *Schistosoma haematobium* infection were followed up quarterly to determine infection and morbidity incidence.

Results At baseline, the prevalence of *S haematobium* infection and microhaematuria was 8.5% and 8.6%, respectively. Based on different anthropometric indices, 2.2%–8.2% of children were malnourished, 10.1% underweight and 18.0% stunted. The fraction of morbidity attributable to schistosome infection was 92% for microhaematuria, 38% for stunting and malnutrition at 9%–34%, depending on indices used. *S haematobium*-positive children were at greater odds of presenting with microhaematuria (adjusted OR (AOR)=25.6; 95% CI 14.5 to 45.1) and stunting (AOR=1.7; 95% CI 1.1 to 2.7). Annual incidence of *S haematobium* infection and microhaematuria was 17.4% and 20.4%, respectively. Microhaematuria occurred within 3 months of first infection and resolved in a significant number of children, 12 weeks post-praziquantel treatment, from 42.3% to 10.3%; P<0.001.

Conclusion We demonstrated for the first time the incidence of schistosome infection in PSAC, along with microhaematuria, which appears within 3 months of first infection and resolves after praziquantel treatment. A proportion of stunting and malnutrition is attributable to *S haematobium* infection. The study adds scientific evidence to the calls for inclusion of PSAC in schistosome control programmes.

Key questions

What is already known about this topic?

- Epidemiological studies indicate that preschool-aged children (PSAC) aged ≤5 years are exposed to schistosome infection, the consequences of which manifest later in life.
- Unlike school-aged children, there are no longitudinal studies tracking the dynamics of new infections, the development of morbidity and implications on current and future health in this age group.

What are the new findings?

- We determined for the first time levels of schistosome morbidity in PSAC attributable to *Schistosoma haematobium* infection, that is, 92% of microhaematuria, 38% of stunting and depending on what index is used, 9%–34% of malnutrition.
- We recorded significant annual incidence of new schistosome infection (17.7%) and urinary morbidity (microhaematuria; 20.4%) with significant quarterly incidences.
- We showed that microhaematuria occurred within 3 months of first infection and resolved after praziquantel (PZQ) treatment.
- We indicated that a significant amount of morbidity, as measured by microhaematuria, resolved within 3 months of effective treatment with PZQ (significant reduction from 42.3% vs to 10.3% (P<0.0001)).

Recommendations for policy

- The findings indicate that schistosome morbidity in PSAC can be reversed by PZQ treatment.
- The findings contribute to the scientific evidence base for prioritising schistosome treatment in PSAC, to reduce infection and morbidity and promote child health and development.

INTRODUCTION

Of the 123 million children worldwide affected with schistosomiasis, about 50 million are preschool-aged children (PSAC), that is, ≤5 years.[1] Nonetheless, schistosome infection and morbidity dynamics in this age group are less characterised compared with school-aged children (SAC), that is, ≥6 years. For example, there are several studies describing and quantifying schistosome-related morbidity including haematuria, nutritional deficiencies and delayed growth and cognition in SAC[2 3] but no comparable comprehensive studies in PSAC.

Epidemiological studies in PSAC clearly indicate that infection occurs in early childhood,[4–7] and if untreated, the infection can lead to health consequences later in life.[8] Despite this importance of childhood infections, there is a paucity of longitudinal studies tracking the dynamics of new first infections, the development of morbidity and implications on current and future health in this age group. The definition of schistosome pathology and morbidity continues to be refined in attempts to better characterise clinical manifestations, for example, as with female genital schistosomiasis,[9] and to identify applicable morbidity markers of disease, for example, urine albumin–creatinine ratio.[10] Growth and nutrition-related morbidities associated with schistosomiasis have also only recently become more widely recognised.[11] There is therefore a need to collate all of this new knowledge to better define schistosomiasis in PSAC, where manifestations of disease are poorly described.[6]

In this study, we aimed to describe the baseline dynamics of schistosome infection and morbidity in Zimbabwean PSAC exposed to *Schistosoma haematobium*. A cohort of schistosome-negative children was followed for a year to document infection and morbidity incidence, as well as the effects of treatment on infection and morbidity. This study investigates the ability of existing diagnostic and morbidity tools to quantify and monitor early infection and morbidity. It also contributes to disease burden estimates and the dynamics of infection and morbidity in PSAC. This knowledge will inform the design and implementation of interventions targeted at this age group.

METHODS

Consent

Permission to conduct the study in the province was obtained from the Provincial Medical Director. Prior to enrolment, the study aims and procedures were explained to all participants and their parents/guardians in English or in the local language, Shona. Written informed consent was obtained from the participants' parents/guardians as appropriate. Recruitment into the study was voluntary and parents/guardians were free to withdraw the participants at any time with no further obligation.

Study site and period

The study was conducted in 13 villages in the Shamva district, northeast of Zimbabwe (17°10′0″S 31°40′0″E) from February 2016 through to February 2017. This is one of seven districts in the Mashonaland Central province of Zimbabwe, whose people are primarily subsistence farmers. There is a cold dry (April–July), hot dry (August–October) and rainy season (November–March).[12 13] The area was selected for this study on urogenital schistosomiasis because the prevalence of *S haematobium* is high (>50%), while the prevalence of *Schistosoma mansoni* and soil-transmitted helminths is low (<15%).[14]

Study design

This study was part of a larger longitudinal parasitological and immunological project, following the treatment-re-infection study design widely used in human helminth field studies. There was a cross-sectional study at baseline, followed by a 1-year longitudinal study. Recruited children were screened at baseline for schistosome infection and morbidity to describe the epidemiology of infection and morbidity in this population. The larger study is comparing the impact of regular quarterly screening and treatment (group 1) and biennial screening and treatment (group 2). This study reports on the findings in the children screened quarterly (group 1). Following the baseline recruitment, age and sex-matched *S haematobium*-negative children who fulfilled the inclusion criteria were randomly allocated into groups 1 and 2. A total of 1783 children were invited to participate, of which 1502 provided samples for parasitological diagnosis at baseline. After allocation to the two groups of the study, 525 children who were schistosome-negative by egg count, provided a blood sample for serological assays and consented to participate in the longitudinal follow-up, formed the group 1 cohort, which was followed up every 3 months to detect new schistosome infections by egg count, and morbidity by microhaematuria.

Children were recruited from crèches, early child development centres, and preschools. Parents/guardians of children not attending any of the educational programmes (eg, children <3 years) were invited through the community nurse and village health workers to report to the sampling centre; that is, the centre used by the community for the Expanded Program for Immunisation (eg, school or primary health centre) for enrolment into the project. A questionnaire designed in English and translated into the local dialect (Shona) was administered to gather demographic data and establish exposure behaviour.

Study inclusion criteria

At baseline, the study enrolled children aged 6 months to 5 years who met the following inclusion criteria. Participants had to (i) be lifelong residents of the study area, (ii) have no previous antihelminthic treatment, (iii) be negative for *S mansoni* and (iv) consent to participate. To be included in the longitudinal cohort, children who had fulfilled the inclusion criteria described above had to meet an additional criterion of being diagnosed negative for *S haematobium* by egg count at baseline.

Anthropometry

Weight (nearest 0.1 kg) and height (nearest 0.1 cm) without shoes and in light clothing was measured using an electronic scale and a stadiometer, respectively. For very young babies, height was measured with an infantometer baby board, and weight measured with a baby scale. The mid-upper arm circumference (MUAC) was measured (nearest 1 mm) using a child MUAC tape; on the left arm, midpoint between the shoulder and the tip of the elbow, with the arm relaxed and hanging down the body. Growth and nutritional status was assessed using the WHO Anthro software V.3.0.1 (http://www.who.int/childgrowth/en/).[15] This generated Z-scores for specific measures of nutrition and growth, that is, stunting by height-for-age (HAZ), underweight by weight-for-age (WAZ) and body mass index-for-age (BAZ) and malnutrition by MUAC (MUAC and MUACZ) and weight-for-height (WHZ). Measures were considered abnormal when Z scores were <–2.[16]

Parasitological diagnosis

About 50 mL of urine sample was collected from each participant on three successive days and a stool specimen was collected on a single day from each participant. Samples were collected between 10:00 hours and 14:00 hours, and processed within 2 hours of collection. For very young children, urine bags (Hollister 7511 U-Bag Urine Specimen Collector, Hollister, Chicago, Illinois, USA) and disposable diapers were used to collect urine and stool samples respectively. Urine samples were examined microscopically for *S haematobium* infection following the standard urine filtration method[17] and the number of eggs was reported per 10 mL of urine. Stool samples collected were processed using the Kato–Katz method[18] and parasite eggs enumerated under a light microscope for *S mansoni* (in duplicates) per gramme of stool.

Children were diagnosed positive for helminth infection if at least one parasite egg was detected in their urine or stool samples. All children who were positive for *S haematobium* infection were treated with a single dose of praziquantel (PZQ) at the standard 40 mg/kg bodyweight at each visit. Tablets were crushed and administered with squash and sliced bread[19] by local nurses. A post-treatment efficacy check (egg count) was carried out for all such participants at each subsequent follow-up (12 weeks post treatment).

Detection of urinary morbidity

Urine samples collected were examined for visible haematuria (macrohaematuria). Microhaematuria was determined by dipping the reagent end of Uristix reagent strips (Uripath, Plasmatec, UK) into fresh, well-mixed urine for 40 s and the test area compared with a standard colour chart as per manufacturer's instructions. The strength of the colour change indicates varying concentrations of blood present in the sample, that is, negative, trace, positive (+), positive (++), positive (+++) and positive (++++). For analysis purposes, microhaematuria was classified as either negative or positive.

Sample size calculations

The sample size used for this study was based on the larger, longer-lasting study comparing reinfection rates following different treatment regimens, of which this is a subset. Calculations were based on previous studies showing that PZQ treatment reduces reinfection rates by at least 50%.[5 14] Statistical power analysis was performed using Gpower V.3.1.5.[20] Based on an expected prevalence of 6.7%, as derived from our previous study in children aged 1–5 years,[21] a sample size of 214 in each group would provide α=0.05 and power=0.80. Allowing for a 20% dropout rate, the sample size required for each group during follow-up was 256. The sample size for the larger reinfection study, that is, 525 in a group, was therefore sufficient for this aspect of the study.

Statistical methods

Data analyses were performed using SPSS V.22 (IBM) and GraphPad Prism V.7.02 (GraphPad Software). The χ^2 (or Fisher's exact test for small sample sizes) and the Mann-Whitney was used to test for differences in categorical and numerical variables, respectively. Infection intensity for *S haematobium* was defined as the arithmetic mean egg count/10 mL of at least two urine samples collected on three consecutive days. The egg count data was further log-transformed ($\log_{10}$ [egg count+1]) to meet the normality assumption of parametric statistics. At baseline, infection intensity (log-transformed) and its relationship as a function of age was determined using a linear regression model. To determine the infection prevalence based on a binary response as a function of age, logistic regression modelling was used as previously described.[22] The effect of different factors on the prevalence of schistosome infection and morbidity was determined using logistic regression and the results reported as adjusted ORs (AORs) and 95% CI, along with the test for significance.

For a given morbidity marker, the attributable fraction (AF) in the exposed population (*S haematobium*-positive) and in the total population was calculated using Miettinen's formulae[23]:

$$\text{AF in the exposed population } (AFe) = \frac{(RR-1)}{RR}$$

$$\text{AF in the total population } (AFp) = Pe \times AFe = Pe \times \frac{(RR-1)}{RR}$$

In the formulae, RR is the risk ratio of morbidity associated with exposure, Pe is the prevalence of morbidity among the exposed (*S haematobium*-positive). Because the AFs in this case are from a helminth study and estimated at the cross-sectional level, we substituted the RR with prevalence ratios (PR) as recommended in helminth epidemiology.[24] The PR was estimated as a ratio of the proportion of infected individuals with morbidity to the

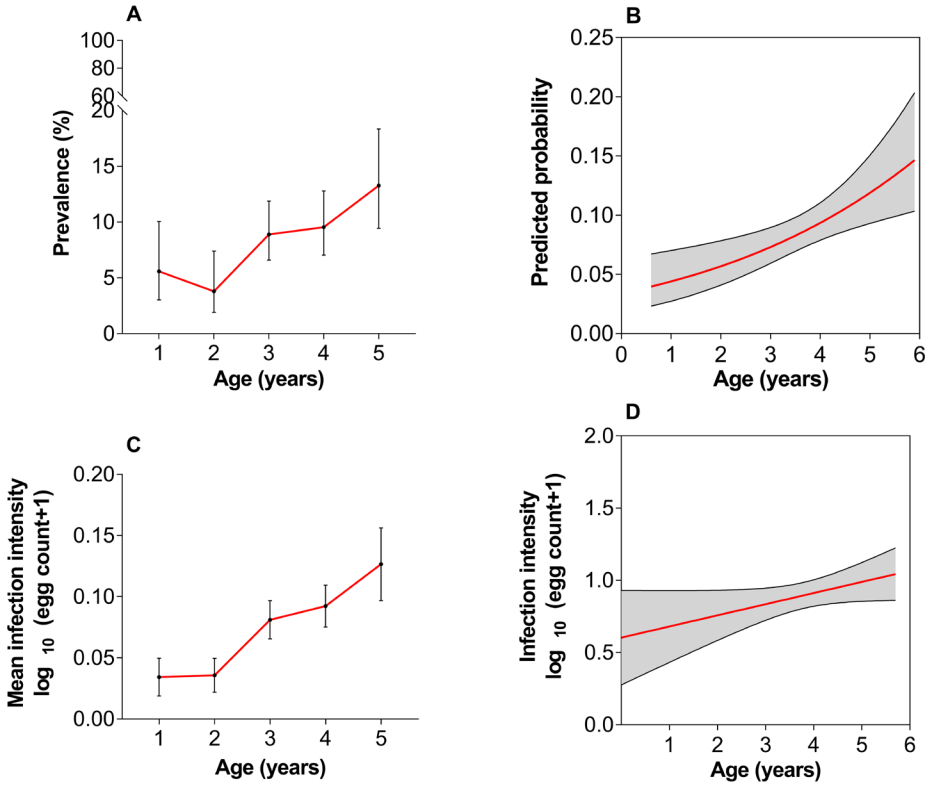

Figure 1 (A) *Schistosoma haematobium* infection prevalence with age; prevalence varied with age (P<0.001) and (B) age-predicted probability of infection; prevalence increased as children grew older (P=0.002). (C) *S haematobium* infection intensity with age; intensity varied with age (P<0.001) and (D) age-predicted intensity of infection; infection intensity increased as children grew older. Error bars indicate 95% CI (A) or SEM (C), and shaded areas indicate 95% CI; (B, D).

proportion of uninfected individuals with morbidity. AFs were calculated on morbidity markers with PR>1, suggesting an increased risk of morbidity from schistosome infection.[24] Treatment efficacy was assessed by means of egg reduction rates (ERR) and cure rates (CR) as described previously.[21]

Approximate CIs were calculated using the modified Wald method[25] and P<0.05 was considered significant.

RESULTS

Demographics

Of the 1502 recruited into the study, 794 (52.9%) were male. Age range was between 0.5 and 5 years (median=3.5 years; IQR 2.5–4.3). The youngest participant in whom *S haematobium* infection was detected was a year old. Maximum loss to follow-up was recorded at first follow-up in May 2016 (64 participants; 12.2%). Overall follow-up rates in the longitudinal cohort, including participants for post-treatment efficacy check, were 87.8% in May 2016, 93.7% in August 2016, 95.1% in November 2016 and 88.8% in February 2017.

S haematobium epidemiology at baseline

S *haematobium* infection prevalence at baseline in the 1502 participants was 8.5% (95% CI 7.2 to 10.0). The median age of children positive for infection was significantly higher compared with those negative for infection (4.0 vs 3.5 years; P=0.001). Infection prevalence increased with age as shown in figure 1A,B. The overall mean infection intensity was 7.9 eggs/10 mL urine (95% CI 6.4 to 9.7). The majority of children, 93.7% (95% CI 87.9 to 97.0) presented with light infections (<50 eggs/10 mL of urine) based on the WHO classification.[19] Infection intensity increased with age as shown in figure 1C,D. There was no significant difference in infection prevalence between males and females; 8.9% (95% CI 7.1 to 11.1) and 8.1% (95% CI 6.3 to 10.4; P=0.067), respectively.

Morbidity at baseline

Prevalence of urinary morbidity was 0.7% (95% CI 0.3 to 1.5) for macrohaematuria and 8.6% (95% CI 6.9 to 10.6) for microhaematuria. Malnutrition measured by different indices were as follows: MUAC, 2.2% (95% CI 1.4 to 3.2), MUACZ, 7.4% (95% CI 6.0 to 9.1) and WHZ, 8.2% (95% CI 6.8 to 9.9). Prevalence of underweight measured by WAZ was 10.1% (95% CI 8.5 to 11.9), and stunting by HAZ was 18.0% (95% CI 16.0 to 20.3). Comparing infected versus uninfected children, prevalence of microhaematuria (43.5%; 95% CI 34.8 to 52.6 vs 3.4%; 95% CI 2.4 to 5.0; P<0.001) and stunting (27.0%; 95% CI 19.9 to 35.6 vs 17.0%; 95% CI 14.9 to

Table 1 Prevalence ratios (PRs) for detected schistosome-related morbidity

Morbidity	Diagnostic tool	PR (95% CI)
Microhaematuria	Urine dipsticks	12.6 (11.6 to 14.1)
Macrohaematuria	Visual inspection (colorimetry)	3.4 (1.9 to 5.4)
Stunting	HAZ	1.6 (1.05 to 2.31)
Malnutrition	WHZ	1.1 (0.9 to 1.4)
	MUACZ	1.5 (1.3 to 1.9)
	MUAC	1.3 (0.8 to 1.9)
Underweight	WAZ	1.4 (1.2 to 1.6)
	BAZ	1.0 (0.8 to 1.3)

BAZ, body mass index for age Z scores; HAZ, height-for-age Z scores, MUAC, mid-upper arm circumference Z scores; WHZ, weight-for-height Z scores; WAZ, weight-for-age Z scores.

19.4; P=0.009) was significantly higher among children with *S haematobium* infection.

Morbidity attributable to *S haematobium* infection

Morbidities from schistosome infection are not specific and may relate to different physiological, biochemical and immunological processes. We determined how much of the detected morbidity was attributable to schistosome infection by first determining PRs. All the morbidity markers considered had PR >1 (significant association with schistosome infection) except underweight by BAZ (table 1). Based on AFs, microhaematuria was the most dominant morbidity marker attributed to schistosome infection both in infected children and at the population level. Macrohaematuria, on the other hand, was highly attributable to schistosome infection in the infected population but this was not the case in the total population. Of the anthropometric markers, stunting was the most dominant marker attributed to schistosome infection both at the population level and among the infected children (figure 2).

Likelihood of schistosome infection and morbidity

Multiple logistic regression analysis showed that with every unit increase in age, children were more likely to acquire *S haematobium* infection (AOR=1.4; 95% CI 1.1 to 1.8; P=0.005). Children who presented with microhaematuria were more likely to be positive for *S haematobium* infection (AOR=21.8; 95% CI 11.7 to 40.7; P<0.001) as shown in figure 3A. Similarly, children presenting with *S haematobium infection* were more likely to present with microhaematuria (AOR=25.6; 95% CI 14.5 to 45.1; P<0.001) and stunting (AOR=1.7; 95% CI 1.1 to 2.7; P=0.014) as shown in figure 3B.

Incidence of infection and morbidity

To determine infection and morbidity incidence, 525 schistosome-negative children were followed quarterly for 12 months to determine schistosome infection and morbidity acquired in the previous three months. Based

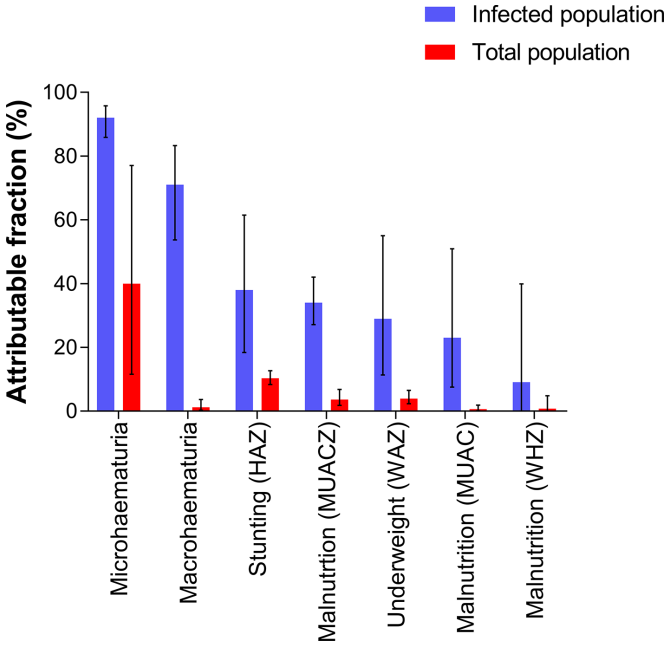

Figure 2 Estimated proportion of morbidity attributable to *Schistosoma haematobium* infection in the infected population (blue; AFe) and in the total population (red; AFp). Error bars indicate 95% CIs. BAZ, body mass index-for-age Z scores; HAZ, height-for-age Z scores; MUAC, mid-upper arm circumference Z scores; WAZ, weight-for-age Z scores; WHZ, weight-for-height Z scores .

on the longitudinal data, annual incidence of *S haematobium* infection was 17.4% (95% CI 13.7 to 21.8) and that of microhaematuria was 20.4% (95% CI 15.8 to 26.0). *S haematobium* incidence rates in the dry season was 4.9% in May (95% CI 3.1 to 7.8) and 6.5% in August (95% CI 4.1 to 9.9) while that in the rainy season was 3.8% in November (95% CI 2.1 to 6.6) and 3.7% in February (95% CI 2.1 to 6.5). Difference in overall incidence rates however was not significant between the dry (10.4%; 95% CI 7.6 to 14.1) and rainy seasons (7.4% total; 95% CI 5.0 to 10.9; P=0.175). The quarterly incidence of microhaematuria recorded was 2.0% in May (95% CI 0.4 to 5.9), 2.8% in August (95% CI 1.0 to 6.6), 13.3% in November (95% CI 9.6 to 18.3) and 4.3% in February (95% CI 2.2 to 8.1).

Treatment efficacy and effects on morbidity

The treatment efficacy was calculated from children positive for infection at baseline and those that became infected throughout the year. Thus, a total of 187 children were treated for infection (127 at baseline and 60 from the longitudinal cohort), of which post-treatment data were available for 156 (follow-up rate: 83.4%). PZQ was efficacious in reducing *S haematobium* infection, as indicated by the high CR (96.2%; 95% CI 91.7 to 98.4) and ERR (99.8%; 95% CI 99.2 to 100). In addition, the mean infection intensity pretreatment (7.1 eggs/10 mL urine; 95% CI 5.9 to 8.6) was significantly reduced at post-treatment follow-up (1.1 eggs/10 mL urine; 95% CI 1.0 to 1.2; P<0.001).

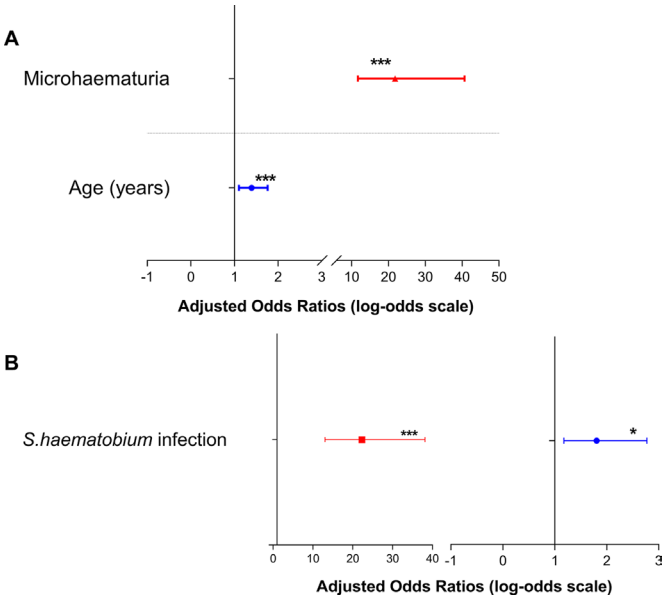

Figure 3 Forest plot showing (A) the odds of presenting with *Schistosoma haematobium* infection and (B) odds of presenting with microhaematuria (left) and stunting (right). Error bars indicate the 95% CIs. *P<0.05, ***P<0.001. Non-significant variables were excluded from the final logistic regression model.

To determine the effects of treatment on morbidity identified at baseline, data for microhaematuria were available for 78 of the 127 S *haematobium*-positive cases identified. Within this cohort, 42.3% (95% CI 32.0 to 53.4) were positive for microhaematuria and this declined significantly post-treatment (10.3%; 95% CI 5.1 to 19.2; P<0.001).

A pooled analysis of participants in whom new infections were detected throughout the follow-up period (group 1) was done to determine the dynamics of microhaematuria with infection, before, during and post infection. A total of 60 new infections were detected throughout the follow-up period. Of this, microhaematuria was detected among 18 individuals; 6 (33.3%) preinfection, 11 (61.1%) during infection and 2 (11.1%) post treatment. In 61.1% of these individuals, microhaematuria coincided with the detection of *S haematobium* infection (within 3 months) and had resolved by the next survey at 3 months post treatment of infection (figure 4).

DISCUSSION

Contrary to the previously held assumption of low risk to schistosome infection in PSAC,[26] the frequency of schistosome infections among infants and young children is being increasingly recognised.[27] We conducted a longitudinal study in a cohort of Zimbabwean PSAC to determine the prevalence, dynamics and incidence of first urogenital schistosome infection and morbidity and its associated risks and health impacts. We showed that PSAC present with schistosome infection (estimated by egg count) and associated morbidity (determined by microhaematuria, growth and nutritional markers) from an early age. We also found that schistosome infection and morbidity can be detected early in PSAC using parasitology and microhaematuria within 3 months of infection and is resolved after treatment.

The observed baseline prevalence of *S haematobium* infection in PSAC (8.5%) is comparable to levels recorded in PSAC in Ghana (11.2%),[4] Malawi 10.7%[28] and in our recent studies in Zimbabwe, that is, 13.5%[10] and 6.7%.[21] PSAC however present with light infections[22 27 29] and parasitological egg counts underestimate the prevalence of schistosome infection.[4 22] We anticipate this prevalence to increase if the more sensitive serological diagnostic tools are used.[22] In agreement with previous findings,[7 22 30–32] infection prevalence and intensity increased as children grew older.

The majority of morbidity biomarkers associated with schistosomiasis are non-specific and relate to various physiological, biochemical and immunological processes.[33] We determined the prevalence of morbidity and how much of this was attributable to *S haematobium* infection. Microhaematuria was the most dominant marker for schistosome-related morbidity, and children with *S haematobium* infection were more likely to present with microhaematuria and vice versa. This agrees with our previous findings in Zimbabwe[10] and that by researchers in Nigeria[34] on the significance of microhaematuria as a point of care field marker of morbidity in PSAC.

In addition to biomarkers, we also investigated the prevalence of stunting and malnutrition in the children. To the best of our knowledge, this is the first study to show the relationship between *S haematobium* infection and chronic growth failure (Stunting by HAZ) in PSAC, although studies on polyparasitism[35 36] and few schistosome-specific studies[37 38] have documented this effect in older children. In accordance with our findings, stunting as detected in older children and adolescents is believed to be the result of chronic antiparasite inflammation which persists during childhood.[39] Causality is difficult to establish in this case due to the lag time between the initial infection and the time at which we measured growth failure, and the impact of confounding factors including diet and coinfections. However, there is the need for longer-term studies investigating the impact of treatment on growth and development measures in PSAC. Statistical modelling suggests that with early, repetitive treatment of infection before 6 years of age 'catch-up growth' can be effectively facilitated.[40]

While baseline prevalence and intensity of schistosome infection have been described in PSAC from several African countries, there has not been an incidence study published to date. Here, we document the incidence of urogenital schistosome infection and morbidity in PSAC. Our quarterly incidence is an indication of new schistosome infections in PSAC in endemic areas and the applicability of current tools (urine filtration and urine dipstick) to screen for early infection and morbidity. The incidence of microhaematuria and the AF analyses suggest that even in the very first episodes of infection

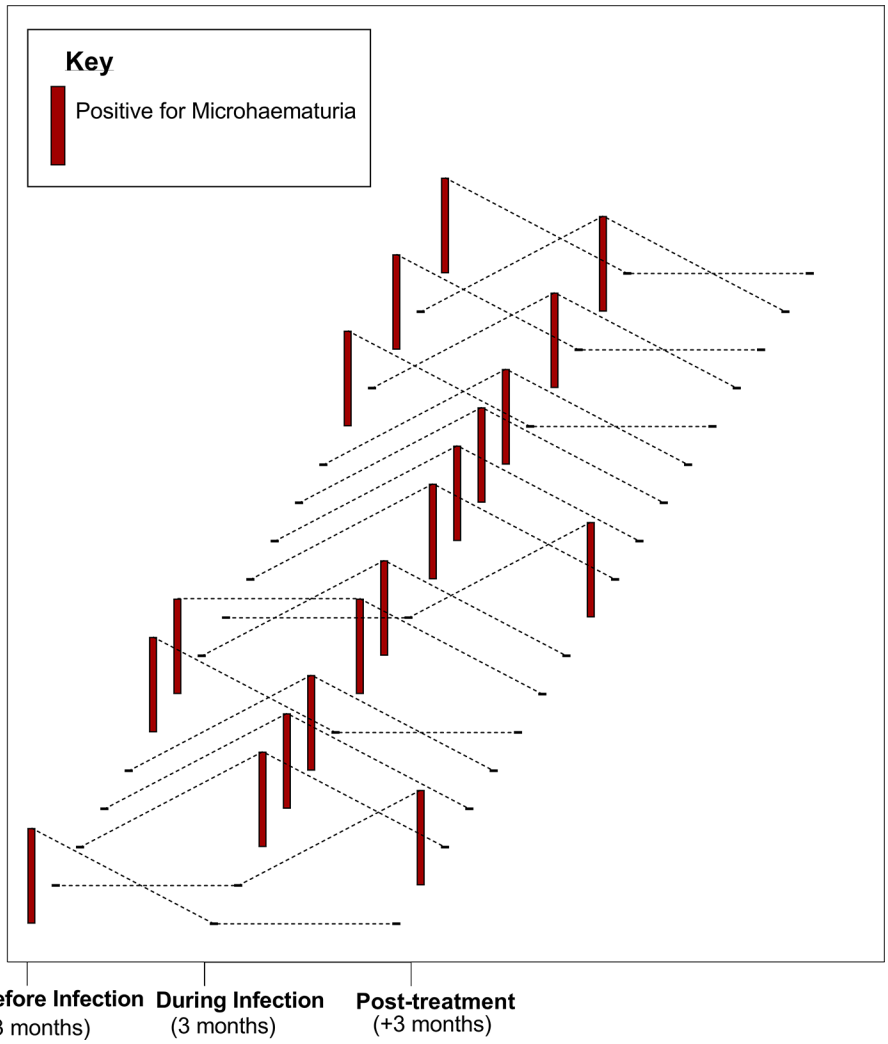

Figure 4 Impact of *Schistosoma haematobium* infection and praziquantel (PZQ) treatment on morbidity (microhaematuria). Microhaematuria status for 18 individual participants is shown at three time points: before, during and post infection (post treatment). Each data set (dotted line) represents one individual. Tall, red bars indicate positive microhaematuria, and a black dash indicates negative microhaematuria at specific time points.

PSAC suffer morbidity, reflected as microhaematuria; an indication of active bladder and ureteral lesions[41 42] and blood loss even in mild schistosome infection.[43]

PZQ, the antihelminthic of choice for treatment of schistosomiasis, is safe and efficacious in PSAC.[44] Our results 12 weeks post treatment showed that a single standard dose of PZQ was effective against *S haematobium* infection. This is consistent with reports on the efficacy of PZQ treatment for schistosomiasis in PSAC.[5] Microhaematuria correlates with *S haematobium* infection,[42 45] and treatment with PZQ reduces morbidity (microhaematuria) as used in large-scale chemotherapy for SAC.[33 46] We observed that microhaematuria occurred rapidly within 3 months of exposure to infection and resolved within 3 months after treatment with PZQ. This is an important indicator for non-delayed schistosome screening and treatment in PSAC, to avert cumulative morbidity which can affect overall health.[1] Observation from our field studies prove that suggestions to empower health workers to screen for infection, and making PZQ available in health centres for treatment on detection will be an important control strategy in this age group.[47]

The seasonal pattern of infection incidence detected is in agreement with the fact that during the dry seasons snail vectors and larval schistosomes become concentrated at permanent and slow-moving water sources, increasing the risk of infection.[48] Our observation from fieldwork also indicates that during the rainy seasons, households are less reliant on water sources for chores and children are less likely to visit water bodies for recreational purposes.

The impact of schistosome infection on the health of children is likely to be greater than those explored here, for example, its impact on neurocognitive development. Mechanistic and epidemiological studies separating the effects of schistosome infections from other confounders would be informative in identifying and portioning causation in AFs. The present study did not measure the impact of existing feeding, nutrition habits and socioeconomic status on stunting and its relationship

to schistosome infections. A limitation of the parasitological detection of infection is that some light infections may have been missed, resulting in underestimation of the infection rates observed. Nonetheless, the study allows comparison with other studies while parasitological methods remain the predominant schistosome diagnostic in PSAC.

CONCLUSIONS

We demonstrated for the first time the incidence of schistosome infection and morbidity in PSAC. We have also shown that a significant proportion of stunting and malnutrition is attributable to *S haematobium* infection. Morbidity assessed by microhaematuria occurs rapidly within 3 months of first infection and resolves post treatment. More importantly for childhood health and development, schistosome treatment leads to a significant decline in microhaematuria and this resolution occurs within 3 months of PZQ treatment. The study adds scientific evidence to the calls for inclusion of PSAC in schistosome control programmes. Non-delayed schistosome screening and treatment in PSAC is essential to avert accumulative morbidity which can affect overall health.

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Contributors DNMO, TM, NM, MEJW and FM were involved in conceptualisation. MEJW and FM designed the study. TM, NM, MEJW and FM were involved in project supervision DNMO, TM, NM, MJM-M, TC, EE, TM, LTP, WMW, SAA, JM, CT and FM conducted the field work. DNMO, TC, EE, LTP and WMW curated the data. DNMO, MCT, WMW, MEJW and FM analysed the data. DNMO and FM prepared the draft manuscript, and all authors were involved in review and editing of the manuscript. All authors read and approved the final manuscript.

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PLOS | NEGLECTED TROPICAL DISEASES

REVIEW

Coinfections and comorbidities in African health systems: At the interface of infectious and noninfectious diseases

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Abstract

There is a disease epidemiological transition occurring in Africa, with increasing incidence of noninfectious diseases, superimposed on a health system historically geared more toward the management of communicable diseases. The persistence and sometimes emergence of new pathogens allows for the occurrence of coinfections and comorbidities due to both infectious and noninfectious diseases. There is therefore a need to rethink and restructure African health systems to successfully address this transition. The historical focus of more health resources on infectious diseases requires revision. We hypothesise that the growing burden of noninfectious diseases may be linked directly and indirectly to or further exacerbated by the existence of neglected tropical diseases (NTDs) and other infectious diseases within the population. Herein, we discuss the health burden of coinfections and comorbidities and the challenges to implementing effective and sustainable healthcare in Africa. We also discuss how existing NTD and infectious disease intervention programs in Africa can be leveraged for noninfectious disease intervention. Furthermore, we explore the potential for new technologies—including artificial intelligence and multiplex approaches—for diagnosis and management of chronic diseases for improved health provision in Africa.

Introduction

The top 10 diseases that account for the most disability-adjusted life years (DALYs) and cause of death in Africa include both infectious and noninfectious diseases, with the amount of DALYs contributed by noninfectious diseases almost catching up to those of infectious diseases [1]. What these data do not indicate is the level of comorbidity within the population, a reflection predominantly of the vertical management of diseases in African countries and a legacy of the historical focus on communicable diseases. In particular, when reporting causes of death, the contribution of comorbidities arising from infectious and noninfectious diseases is not reported. Population studies indicate that several tropical infectious diseases show common epidemiological patterns with age and share risk factors, including poor sanitation and

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lack of safe water [2]. Environmental and socioeconomic factors contribute to the coexistence of these pathogens in the same individual and cause concomitant morbidity [2].

Infectious disease co-occurrence exhibits distinct spatial patterns [3]. This co-occurrence, so-called pathogeographic patterns (Fig 1), observed in sub-Saharan Africa (SSA), overlaps with the distribution of neglected tropical diseases (NTDs) [4] and cancers, directly linked to infections (Fig 1). These NTDs include bacterial, parasitic, protozoal, and viral infections, as per the World Health Organisation (WHO) NTD list from the 10th meeting of the WHO Strategic and Technical Advisory Group for NTDs in 2017 (http://www.who.int/neglected_diseases/diseases/en/), with the most common NTDs being helminth parasites [4]. Helminths have been implicated in several noninfectious diseases including endomyocardial fibrosis [5], hypertension [6, 7], iron deficiency anaemia [8], and cancer [9].

In a recent pilot study, we evaluated a multiplex immunoglobulin (Ig) M and IgG antibody response fingerprinting platform for determining exposure history to pathogens using serum from a Zimbabwean population. Initial analysis showed evidence of recent exposure (IgM) to an array of infections (Fig 2). This platform also allowed the detection of responses to childhood vaccinations, as indicated by the high titres of responses against poliovirus in the child compared to the adolescent and adult. Although this exposure history requires further validation through other diagnostic methods, including parasitology and molecular approaches, it gives an indication of potential coinfections, as already described by others [3, 4].

In addition to diseases arising from infectious pathogens, there is also an increase in chronic noninfectious diseases, including high blood pressure, cardiovascular diseases, diabetes, and cancer. Direct and indirect interactions between infectious and noninfectious diseases have been poorly studied, particularly in African settings. For instance, there are now

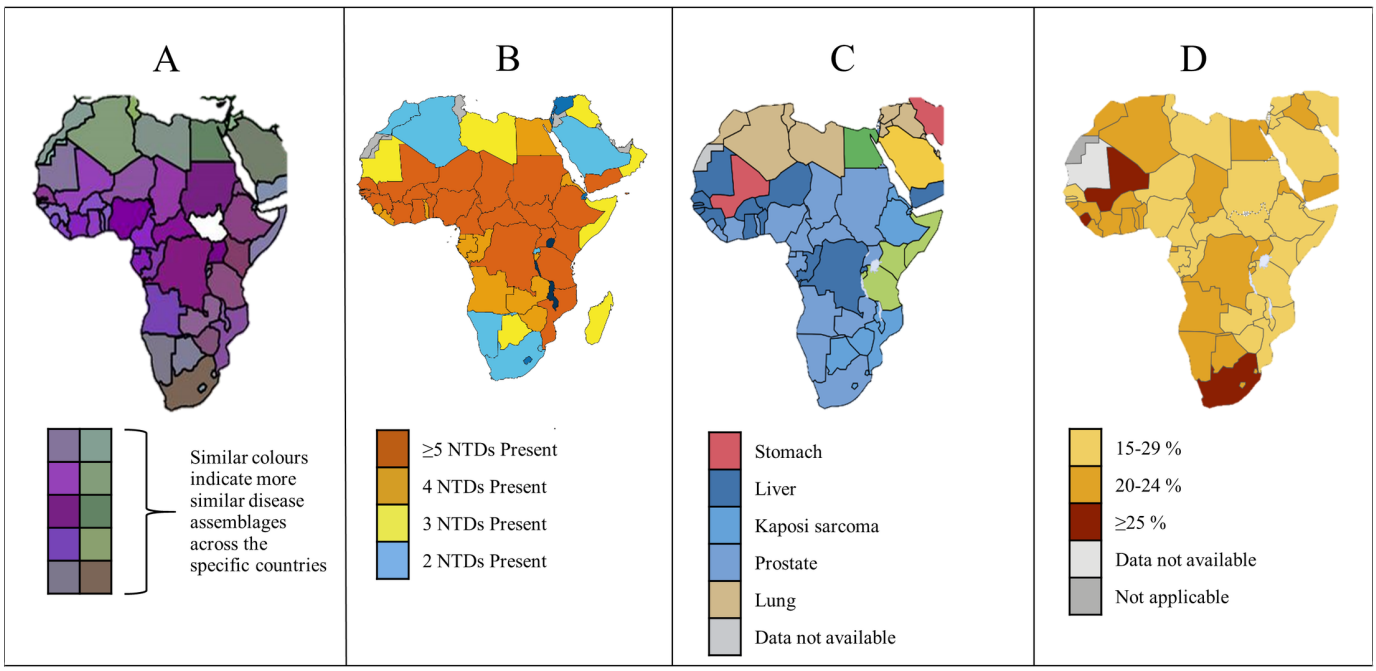

Fig 1. Adapted maps of Africa showing the overlap of neglected tropical diseases (NTDs), infectious, and noninfectious diseases. The figure shows (A) pathogeographic patterns of 187 global human infectious diseases [3], (B) patterns of the six most common neglected tropical diseases [4], (C) burden of the most frequently diagnosed cancer among males [10], and (D) probability of dying from the four main noninfectious diseases between the ages of 30 and 70 years [11]. Infectious diseases show distinct spatial patterns (A), which overlap with the most common neglected tropical diseases (B), commonly diagnosed cancers (C), and the mortality rates from major noninfectious diseases including cardiovascular diseases, cancer, chronic respiratory diseases, and diabetes (D).

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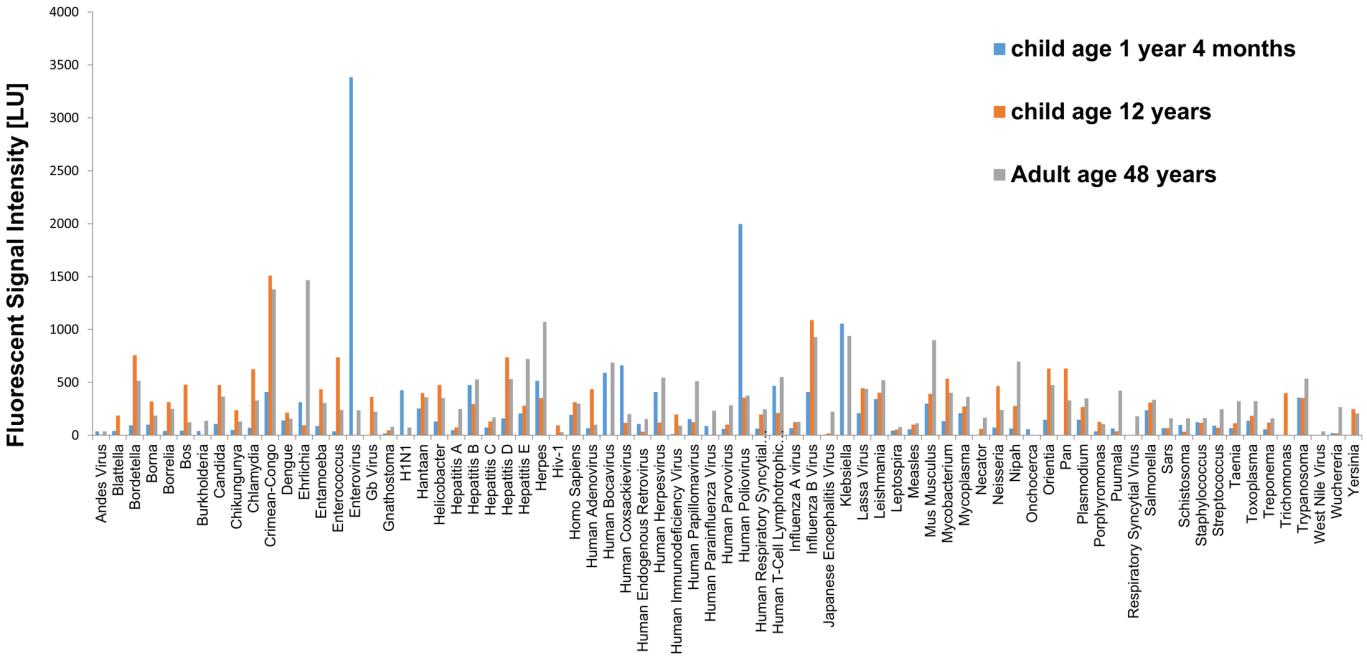

Fig 2. Host infectome analysis based on IgM reactivity to multiple infections in a Zimbabwean cohort. Results indicate variable responses to infections across all age groups. IgM, immunoglobulin (Ig) M.

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suggestions that mental illness may be an inflammatory disease [12, 13], but the sources of inflammation and their relative contribution to mental illness have yet to be determined. Aetiological and mechanistic experimental studies suggest that NTDs, including parasitic infections, may contribute to this inflammation [14]. For example, helminth infection during pregnancy has been suggested to impair neurocognitive development in infants [15], but mechanistic studies have yet to be conducted. Apart from well-known infections, such as human immunodeficiency virus (HIV) and human papilloma virus (HPV) that are risk factors for some cancers [16, 17], there is increasing evidence that inflammation from infectious pathogens contributes to the aetiology of diabetes and coronary artery disease [18, 19].

With increasing coinfections and comorbidities, there is a need to investigate aetiological links between these two groups of diseases (infectious and noninfectious), and to invest in horizontal health systems approaches and training of healthcare workers to manage multiple and chronic conditions. In this review, we hypothesise that the growing burden of noninfectious diseases may be linked directly/indirectly, or further compounded by the existence of NTDs and other infectious diseases. We explore the challenges/barriers to implementing effective and consistent healthcare in SSA in the face of the observed disease trends. We discuss how existing NTDs and other infectious disease intervention programmes and infrastructure can be leveraged for noninfectious disease intervention, diagnosis, and long-term management of diseases, for improved health provision in Africa.

Methodology

A literature review was conducted using electronic databases, including Pubmed/Medline, Google Scholar, and WHO (http://www.who.int). For the entire review, we searched for research articles with keywords relevant to each section of the review. We analysed all articles published and included those relevant to the scope of this review. A systematic review of

literature (PubMed) was done to determine the impact of coinfections in Africa. Search terms for the systematic review were (a) [(Co-infection* OR Coinfection*) AND (Co-morbid* OR Comorbid*) AND (Africa) AND (Health impact*)] (b) [(Co-infection* OR Coinfection*) AND (Co-morbid* OR Comorbid*) AND (Africa) AND (Health impact*) AND Helminth*)]. Selection criteria included human studies, original articles, studies that relate coinfection or comorbidity to a secondary health impact, and articles published in the last 10 years as at January 2018.

Health impact of coinfections

The outcome of coinfections can be asymptomatic, symptomatic, and sometimes fatal. While there are studies—predominantly in experimental models—suggesting the health benefits of infection, e.g., with helminth infections as described through the hygiene hypothesis [20], there are few studies from human populations. These experimental studies may be informative at the mechanistic level, but their phenotypic and thus clinical relevance in humans requires careful and well-designed studies. Animal models of natural infection bridge the gap between experimental and human studies, and these indicate that coinfections can influence population-level disease and mortality patterns, which ultimately influence interventions. For example, a study in African cattle showed that concurrent infection of *Theileria parva* with less pathogenic species of *Theileria* resulted in a reduction in *T*. *parva*-associated mortality [21]. Nonetheless, parasite coinfections in a cattle study showed antagonistic effects that compromised the health of cattle [22].

In human populations, detrimental effects of coinfections have been reported. Polyparasitic infections in Africa have been associated with a higher tendency for wasting, splenomegaly, and anaemia [23]. As shown in Table 1, a systematic review of literature on the health impacts of coinfections in the SSA region suggests the apparent lack of adequate research evidence on the subject matter. The impact of coinfections on health will become more evident as more holistic approaches are taken to studying the health of the host rather than focusing on just pairs of parasite–host relationships or on the interaction and impact of two infections (predominantly HIV and another disease) as has been the predominant practice.

Epidemiology in transition

From acute/episodic diseases to chronic conditions

In addition to communicable diseases, there is an increasing burden of noninfectious diseases such as hypertension, stroke, cancer, and diabetes in the SSA region. This concurrent health challenge is compounded by the lack of marked progress in the control of infection and malnutrition, if at all [42, 43]. The insurgence of noninfectious diseases is a "time bomb" for Africa, with the region expected to record the world's largest increase in noninfectious disease deaths by 2030 [44]. Already, countries in northern and southern Africa account for more than three quarters and close to a half of all deaths to noninfectious diseases, respectively [45].

HIV is now a chronic infection; increased access to antiretroviral therapy (ART) has substantially improved health and reduced the risk of HIV transmission, increasing the life expectancy of HIV patients to one close to that of uninfected populations [46]. Thus, there is an increasing number of over 50-year-old patients living with chronic HIV, and the impact of HIV and ageing on the acquisition of noninfectious diseases like diabetes become key [46], requiring long-term management and care.

Lymphatic filariasis and onchocerciasis pose a serious public health problem in Africa, causing long-term chronic infection with permanent and long-term disability [47]. In human filariasis infections, coinfections with other infectious diseases is common and can affect

Table 1. Summary of publications on health impacts of coinfections in Africa within the last 10 years.

Year	Source	Disease dynamics	Health impacts
2007	Hoffmann and Thio 2007 [24]	Hepatitis B virus–HIV	Liver enzyme alterations, reducing antiretroviral tolerance and increasing its toxic effects. Blunt immune recovery from antiretroviral therapy.
2009	Hadley and Naude 2009 [25]	HIV–Tuberculosis–Malignant tumours	Increased mortality.
	Degarege, Animut et al., 2009 [26]	Malaria–Soil-transmitted helminths	Impact on malaria severity, although small.
2010	lsa, Gwamzhi et al., 2010 [27]	Hepatitis B and C viruses–HIV/AIDS	Impact on causing hepatotoxicity.
	Sangweme, Midzi et al., 2010 [28]	Schistosomiasis–Malaria	Higher peripheral blood malaria parasite density, promoting transmission.
	Modjarrad and Vermund 2010 [29]	HIV–Tuberculosis–Syphilis	Tuberculosis and syphilis may increase HIV viral load, increasing disease progression.
2012	Ntusi, Badri et al., 2012 [30]	*Acinetobacter baumannii*–HIV/AIDS	Increased mortality.
	Faurholt-Jepsen, Range et al., 2012 [31]	Tuberculosis–Diabetes	Poor treatment outcomes including delayed recovery of body mass and haemoglobin levels, hence poor recovery from disease.
	Webb, Barrett et al., 2012 [32]	Chronic myeloid leukaemia–HIV	Poor cytogenic response to leukaemia treatment.
	van den Bogaart, Berkhout et al., 2012 [33]	Visceral leishmaniasis–Malaria	Early detection results in good prognosis, but patients stand a high risk of severe symptoms of leishmaniasis.
2013	Ladep, Agbaji et al., 2013 [34]	Hepatitis B virus–HIV	Reduced survival. With the appropriate treatment Tenofovir, this impact may be annulled
	Taye, Alemayehu et al., 2013 [35]	Podoconiosis–Soil-transmitted helminths	Increased blood losses/anaemia.
2014	Baldassarre, Mdodo et al., 2014 [36]	HIV/AIDS–Cryptococcal meningitis	Increased mortality.
	Knight, Muloiwa et al., 2014 [37]	HIV–Stevens Johnson syndrome–Toxic epidermal necrolysis	Increased risk of systemic bacterial infection and mortality.
	Biraro, Egesa et al., 2014 [38]	Helminths, malaria, or HIV coinfection in household contacts of Tuberculosis patients	No evidence of increased risk to latent Tuberculosis. Th1 cytokine responses in those with prior BCG vaccination was reduced.
	Degarege, Animut et al., 2014 [39]	Malaria–Helminths	Undernutrition; severity is comparable to those with single infections.
2015	Umanah, Ncayiyana et al., 2015 [40]	HIV–Tuberculosis	Treatment failures and increased mortality.
2017	Morawski, Yunus et al., 2017 [41]	HIV–Hookworm	Decreased $CD4^+$ T cell counts during antiretroviral therapy.

Systematic review of literature (PubMed); electronic search terms were (a) [(Co-infection* OR Coinfection*) AND (Co-morbid* OR Comorbid*) AND (Africa) AND (Health impact*)] (b) [(Co-infection* OR Coinfection*) AND (Co-morbid* OR Comorbid*) AND (Africa) AND (Health impact*) AND Helminth*)]. Selection criteria: human studies, original articles, and studies that relate coinfection or comorbidity to a secondary health impact published in the last 10 years.

Abbreviations: AIDS, acquired immune deficiency syndrome; BCG, Bacillus Calmette–Guérin; $CD4^+$, cluster of differentiation 4.

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protective immune responses for infections like malaria and tuberculosis (TB) [48]. Chronic long-term management of filarial infections thus become a very important component of healthcare. This is crucial, especially to prevent secondary infections that may worsen late-stage diseases.

While there are many risk factors associated with the growing number of cancers in Africa, infectious diseases play a significant role (Fig 3). About a third of new cancers in Africa are due to viral, bacterial, or parasitic infections [49]. The implication of this increasing comorbidity of cancer and infectious diseases in Africa means that disease screening, diagnosis, treatment, and care need to be revised to determine potential multiple interventions.

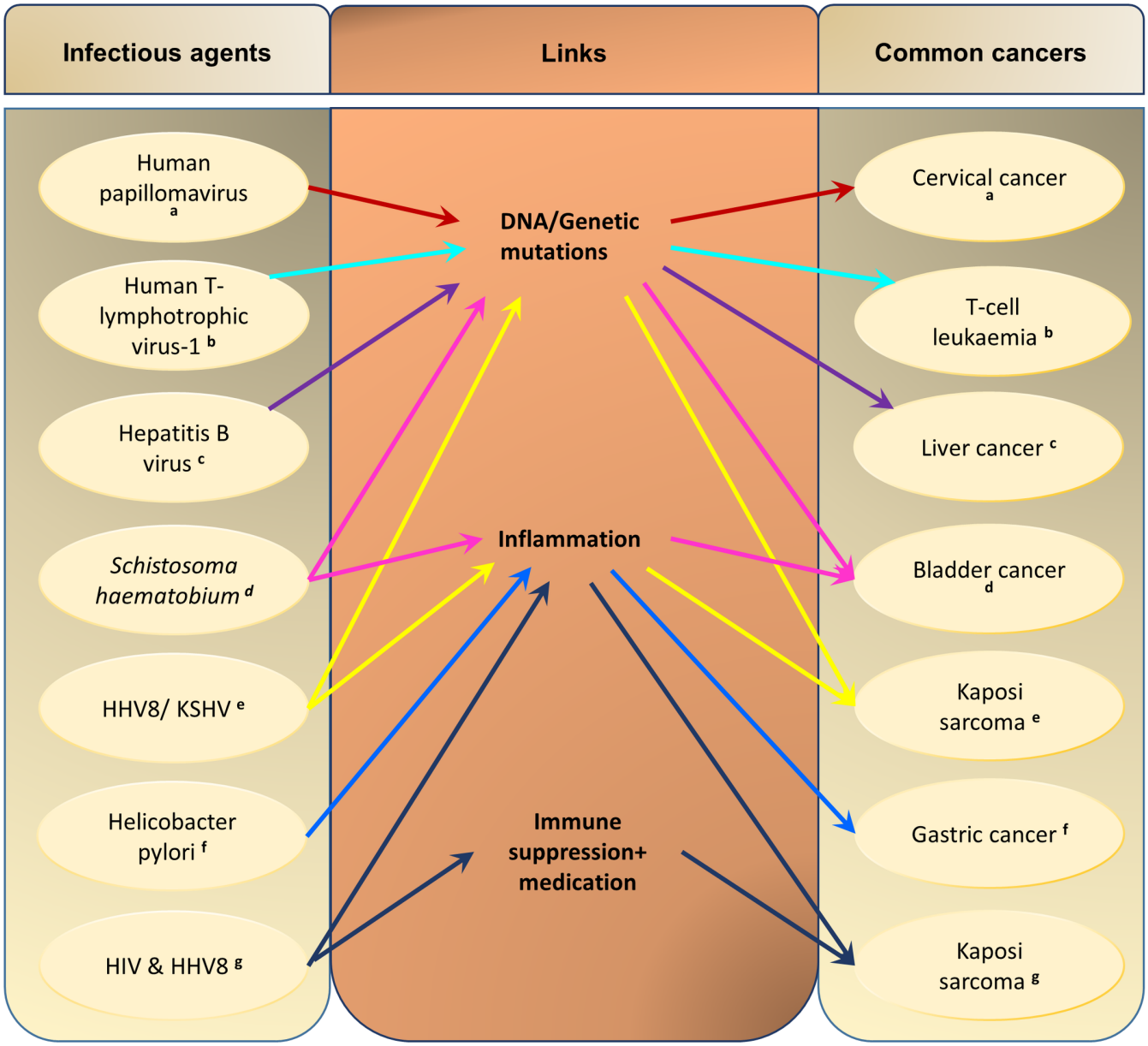

Fig 3. Summary of infections and the types of cancers they cause, via direct or indirect links. Each coloured line/alphabet represents a pathological pattern. Information adapted from [a]Crosbie, Einstein, and colleagues, 2013 [16]; [b]Ahmadi Ghezeldasht, Shirdel, and colleagues, 2013 [55]; [c]Marra, Sordelli, and colleagues, 2011 [56]; [d]Mostafa, Sheweita, and colleagues, 1999 [57]; [e]Dittmer and Damania, 2016 [58]; [f]Polk and Peek, 2010 [59]; and [g]Bower, Nelson, and colleagues, 2005 [17]. HHV8, human herpes virus 8; KSHV, Kaposi sarcoma-associated herpesvirus; HIV, human immunodeficiency virus.

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Developing countries bear over 80% of the global cardiovascular disease burden [50]. At the same time, although previously rare, diabetes has emerged as an important noninfectious disease in SSA [51]. Such metabolic diseases are currently affecting individuals at a much younger age than when compared to developed countries [52]. While individualised approaches to preventing nutritional and metabolic disease can be effective in developed countries, they are not affordable or feasible for the poorer population in low-income countries.

Therefore, societal approaches such as those that have been used in public health educational/awareness campaigns for infectious diseases, notably HIV, will have to be implemented.

The socioeconomic and cultural environment around this current epidemiologic situation in SSA differs from that in most Western countries. In SSA, noninfectious diseases were not anticipated, were accompanied by cultural misconceptions, and have historically received less attention and health budget allocation compared to communicable diseases [53]. Chronic diseases will require long-term management, incurring a cost for both the individual and an already overburdened healthcare system. However, healthcare systems in SSA are designed to provide more acute care, and many are ill-equipped to provide long-term care for chronic conditions, as exemplified in Tanzania [54].

Problems arising from coinfection and comorbidity

The healthcare system. Despite some differences among health systems across Africa, these exhibit some similar structural and organisational formats. Table 2 summarises the general nature and challenges associated with national health systems across Africa by using model countries in different regions of the continent.

The coexistence of multiple infectious and noninfectious diseases, characterised by multiple comorbidities, presents unique problems for healthcare delivery in SSA. From the simulated

Table 2. Health systems in Africa: Structure and challenges.

Region	Model country	System structure	Challenges	Source
Anglophone	Tanzania	Bottom–up approach. Village health services for remote areas at level 1. Level 2 consists of dispensary services for localities with larger populations. Level 3 offers services to even larger populations, up to 50,000 people.	Lack of access for the poor due to the copayment system, insurance requirements, and the insurgence of private physician practices. Absenteeism, low morale, inadequate qualified work force, lack of equipment and supplies. Centralisation at the high level of care.	[63, 64]
	Kenya	Well organised and pyramidal, with dispensaries, health centres, subdistrict hospitals/private clinics, provincial and national hospitals.	Recurrent strikes by doctors, problems with financing health systems, high cost of health services, HIV/AIDS and malaria alone consumes the greatest part of resources.	[65]
	Uganda	Village health teams and community medicine distributors at level 1. Higher up is the health centre II in parishes, health centre III in sub-country, health centre IV, the regional referral hospitals, and three national referral and teaching hospitals.	Village volunteers can be unreliable, lower levels are quick to refer cases. Inadequate infrastructure, inequity in health services, lack of sustenance, low remuneration for staff, paucity of specialised physicians, poor training, high rates of staff layoffs. Poor data collection and utilisation.	[66]
Francophone	Cote d'Ivoire	Follows the 1996 health system organisation with three-tier pyramidal structure. Level 1: health, urban medical, school and university health centres. Level 2: general, regional and specialised hospitals. Level 3: specialised health institutes.	Low level of qualified personnel (one doctor per 10,000). High cost of universal healthcare led to its abandonment, hence lots of out of pocket care.	[63]
	Senegal	Similar structure to that of Cote d'Ivoire. Pyramidal with three levels. Central level: Ministry of Health. Regional level: local health systems. Peripheral level: health districts.	Disparities in distribution of facilities across the country. Sustained by government budget and relies a lot on donor support. Inadequate workforce, inadequate training, poor infrastructure and communication machinery. Social and religious barriers with disparities in quality of care.	[63]
Lusophone	Angola	Has three levels. Primary level: referral health centres or district hospitals, health posts. Secondary care: specialised facilities and general hospitals. Tertiary care: specialised health facilities and central hospitals	Lack of proper remunerations, inadequate allocation of resources by leadership, lack of decentralisation, persistent shortage of essential drugs, lack of data collection and availability.	[67]
	Mozambique	Has four levels. Primary level: health posts (the least equipped) and health centres. Secondary level: rural hospitals and urban hospitals. Tertiary level: five general and seven provincial and district hospitals. Quaternary level: three central hospitals.	Shortage of qualified staff to brain drain, and the system has some of the lowest salaries in Africa. Over reliance on foreign donor support makes it unsustainable. Poor infrastructure and absence of diagnostic tools. Inequitable distribution of health facilities.	[68]
Hispanophone	Equatorial Guinea	Similar structure to that of other countries with a national Ministry of health, Tertiary, Secondary, and Primary healthcare facilities.	Poor leadership and governance, low health financing (93.5% of health cost is out of pocket). Poor service delivery, lack of skilled physicians, and poor management of medical resources. Lack of available health data countrywide.	[69]

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global economic output losses of US$47 trillion from noninfectious diseases over the next few decades [60], low- and middle-income countries are unlikely to be equipped to bear most of this burden. Lessons from chronic HIV management suggest that identifying infected individuals, creating access to therapy, and addressing the multiple complications associated with long-term care requires a well-resourced healthcare system [61]. African health systems are geared toward a more episodic regime of healthcare, without capacity to absorb more patients into chronic disease care in an efficient, affordable, and sustainable manner [54]. Multiplex disease management models, differential diagnostic ability, and proper interventions are essential for long-term patient care. The success of HIV management, including the UNAIDS HIV 90:90:90 care continuum [62], relies on adherence to prescribed medicines, long-term follow up of patients, self-management, and behavioural change by patients.

Health systems in Africa need to be strengthened to improve effectiveness and efficiency across both rural and urban areas, reducing resource wastage, tailoring the training of healthcare providers to the needs of the specific population, along with proper compensation for healthcare providers. Currently, the health system across Africa follows different structural and funding models (see Table 2) and lessons can be learnt from the different models to improve on service delivery and accessibility for comorbidities.

Diagnosis

What is the patient suffering from?. The existing infrastructure in most parts of SSA is not equipped for differential diagnosis. Appropriate basic diagnostic tests to support clinical symptoms may be lacking. For example, in Tanzania, less than 50% of patients with severe malaria (based on WHO clinical criteria) were laboratory confirmed [70], and in Ghana, 40% of such patients were confirmed to have bacterial sepsis and not malaria [71]. Conditions like anaemia, which are common in areas coendemic for different pathogens, still have no simple point-of-care diagnostic tests available. In some cases, despite availability of the technology, conditions are still not diagnosed, e.g., in 15% of Kenyan children with a clinical history of anaemia or malaria, haemoglobin levels were not measured [72]. Where there are diagnostic tests available, their utility may be compromised by the lack of a reference standard test, as occurs in childhood peritoneal tuberculosis (CPTB). In a modelling analysis of five different methods, including the sensitive mycobacterial cultures, tests failed to detect almost 40% of CPTB [73]. In addition, there are reports of challenges with quality control and reproducibility, with very few or nonexistent national laboratory guidelines [74]. Most available diagnostic tests are usually validated in Western populations, without guidelines for use in different populations where a different disease ecology exists. A study in a helminth-infected African population showed that routine allergy diagnostics are impaired by IgE antibodies to the carbohydrate epitope galactose-α-Gal, induced by the parasite infection [75]. Hence, some diagnostic tests used in the SSA region may be failing due to not having been developed/optimised for use in polyparasitic individuals or those presenting with comorbidities.

Lack of centralisation of services impacts healthcare delivery, e.g., in HIV management, nonprofit and commercial organisations are operating specialised independent laboratories [74], which leads to an efficient but exclusive vertical system for HIV management in populations affected by other infectious and noninfectious conditions. Indeed, the absence of evidence-based medicine contributes to poor patient outcomes, misdiagnosis in favour of more common illnesses, delayed treatment, and significant morbidity and mortality.

Patient engagement in healthcare. The importance of the role of the patient in healthcare decisions is increasingly and internationally being recognised, particularly in interventions integrating behavioural change [76]. Central to patient engagement is communication, i.e.,

communicating the diagnostic procedure and results, followed by interventions accessible to the patient. In African health systems, context-appropriate communication of the diagnosis to the patient is challenged by poor education and knowledge of the disease process. A study conducted in South Africa showed that within the African cultural context, most patients viewed the definite diagnosis as having been bewitched, associated it with poor prognosis, and barely understood their diagnosis. On the other hand, health workers expressed concern of inadequate training and lack of competence in communicating diagnosis [77]. In rural Cameroon, most patients tend to disagree with the diagnosis, depending on how well they understood explanations given by the provider. Practitioners often do not give appropriate explanations, do not support patients to express their opinions, and tend to show signs of disapproval when patients do [78]. The ability to communicate diagnosis and, in effect, treatment options becomes more important in the context of coinfections and comorbidities. These highlight important obstacles to appropriate patient care and the need to include proper patient–provider communication as part of healthcare delivery in the face coinfections and comorbidities. Knowledge, attitude, and practice (KAP) studies, including those on both infectious [79] and noninfectious diseases [80], indicate that poor knowledge is associated with practices that increase risk of disease or poor disease management.

What has the patient died of?. In addition to establishing a final diagnosis, autopsies relate the cause of death to associated pathologies that may be present, thereby establishing an interaction [81]. This is important in helping health experts find and track outbreaks, routine diseases and hazards, and helps family members be aware of the genetic risk of diseases. In most parts of SSA, issuing a death certificate is not mandatory and full autopsies are rare due to resource constraints and unwillingness of families to have an autopsy performed [82]. This leads to imprecise approaches to determining the cause of death. A major constraint on global health and development is the absence of mortality patterns due to specific diseases, raising questions on how representative available data are, in relation to populations that go uncounted for. Verbal autopsy is used as an alternative low-cost approach to determine cause of death, and WHO has developed international standards for verbal autopsy, revising its use with automated models [83]. This can be improved by combining verbal autopsies with minimally invasive autopsies (MIAs), an initiative endorsed by funding agencies such as the Bill & Melinda Gates Foundation (BMGF) [84]. Already, reports from Mozambique show significant agreement of MIA with full autopsies [85]. In most of these areas, where MIA is likely to be of benefit, infrastructure such as advanced radiology may not be available, and if at all, it may be expensive. Others have suggested that MIA protocols dependent on needle sampling be used in low- and middle-income countries, although its suitability has yet to be determined [86]. Data from this MIA–verbal autopsy system could be informative for improving future verbal autopsy standards and improving viability and cost of large-scale cause-of-death assignments within SSA.

Interventions

What is the desired outcome?. Diagnosis does not mean cure; therefore, advances in diagnostics must be matched with advances in interventions. Interventions must be informed by knowing what the desired outcome is and what tools are required or available to achieve this. For instance, there is need for a definition of what constitutes a healthy or sick African and what constitutes a healthy or weak immune system amidst all the coexisting infections and morbidities. For example, in a Ugandan healthy population, significant disparity has been described in absolute laboratory values when compared to populations outside SSA, suggesting the necessity to develop specific ranges for the African population [87]. Such heterogeneity is

important for contextualising interventions, e.g., initiation of antiretroviral therapy among HIV patients is informed by CD4 cell counts and any immune reconstitution interventions.

What is the most appropriate drug to use?. Administering treatment in populations affected by coinfections and requiring chronic long-term management requires sufficient knowledge of the type and species of infection, drug–drug interactions within specified populations to inform dosage, and the impact on drug resistance and treatment efficacy. For instance, experience from malaria intervention programmes shows that treatment regimen depends on the target species [88], and our recent studies indicated that repeated treatment was required in multi-*Plasmodium* species malaria-infected individuals when compared to individuals with single species infection (Amanfo and colleagues, in prep). Due to high prevalence of some conditions, clinicians may favour clinical diagnosis against laboratory evidence, treating symptoms instead of causes. For example, in malaria endemic areas, fever may not always be malaria [74], and in the advent of rapid diagnostic tests (RDTs), even in hard to reach areas, majority of these tests may come out negative; in 2014, about 142 million suspected cases of malaria tested negative worldwide [89]. With similar tests lacking for other diseases that cause fever, health workers are left in a dilemma and with nothing to offer. In 2016, a high proportion of febrile children in Africa did not receive medical attention due to poor access to healthcare and lack of awareness among caregivers [89]. Building a stronger health system to deal with such challenges is recommended [90]. Ideally, in cases of patients receiving multiple drugs for multiple conditions, drug–drug interactions need to be considered and managed to maximise efficacy while reducing toxicity. For example, in the administration of the antihelminthic Praziquantel along with Albendazole in multiparasitic interventions, the routine coadministration of both drugs may affect the total exposure of Albendazole [91].

Defining the impact of heritable traits on pharmacology and toxicology in African populations is essential for targeted interventions. For example, cytochrome P450 variants impact drug metabolism [92]. The application of pharmacogenetics can allow prediction of drug efficacy or failure in patients before a drug is deployed, saving time and cost from trial and error prescriptions [93] and may indirectly reduce the development of resistance [94]. Although this requires significant investment, it is clear that near-personalised management of HIV patients already occurring in Africa has already set the precedent.

Potential solutions

Leveraging existing platforms within health systems for disease control

Africa can leverage the successes of infectious disease control programmes to address the increasing burden of noninfectious and chronic diseases. These must encompass innovations that include both prevention and healthcare delivery.

Operational approaches

In many African countries, routine healthcare in the community is delivered through Community Health Workers/Volunteers (CHW). These CHWs are helping efforts to achieve universal healthcare at a low cost per person served. They have been trained to support chronic care and long-term interventions such as supporting community engagement and education, mass drug campaigns for NTD interventions, and maintaining compliance to HIV and tuberculosis treatment. For example, in a population with a high prevalence of HIV, Chibanda and colleagues initiated a low-cost "friendship bench" intervention, locally adapted from problem-solving therapy, to manage mental disorders [95]. In context, such interventions can deliver a successful, practical, yet culturally accepted treatment programme for long-term management of cases [96], with indirect benefits for compliance to treatment for HIV. In SSA, programmes

such as these are rewarding for CHWs and can be sustained over long periods at low costs, hence applicable in the context of available poor health systems. Lessons learnt from HIV control in Malawi are being applied through integrating screening for hypertension into HIV care [97], and in Ghana, decentralised community-based hypertension care has been adapted from HIV management [98].

Control of infectious diseases can also be integrated for greater health impact as exemplified by antihelminthic treatment. Schistosomiasis has been linked to malaria infection in children [99], and schistosomiasis treatment in areas where malaria is coendemic has been shown to reduce malaria transmission [100]. Anthelimintic treatment, in addition to killing the parasites, has been shown to restore neurocognitive performance in school children [101, 102].

Of critical importance is the monitoring and evaluation of any changes within health systems, including integrating health service delivery into existing platforms. For example, what impact does the introduction of a new vaccine have on the health system? Does the expanded programme on immunization (EPI) system in affected countries adjust to cope with such impacts or does this create strains in the system?

Optimising current interventions

Current interventions can be optimised to prevent multiple disease conditions. For example, coadministration of the childhood vaccinations against influenza type B, whooping cough, tetanus, hepatitis B, and diphtheria as a single formulation (Pentavalent) [103] increases compliance. There are already indications that some current vaccines can have broad spectrum effects; the Bacillus Calmette–Guérin (BCG) vaccine can boost the immune system to resist multiple infections [104].

Promotion of already existing measures such as extended breastfeeding programmes has a positive impact on childhood disease and pathology. Breastmilk can contribute to bioactive factors of the innate immune system as well as enhance the protective ability of the gastrointestinal tract [105].

Integrating other platforms into health systems for control

Probiotics and disease control. The utility of probiotic therapy in maternal and child health presents great potential for disease prevention and management, and its role has been extensively reviewed [106]. Experimental studies have shown that intranasal probiotics of *Lactobacillus* strains stimulate immune responses in the respiratory tract, offering protection from viral (H1N1) infection [107, 108]. Probiotics have been used to deliver antigens or adjuvants directly to the "unfriendly" gastrointestinal tract in HIV vaccine development [109] and as a potential cryopreservative and immunomodulator of mucosal immune response in Hepatitis B vaccines [110]. Despite existing evidence on the role of probiotics to enhance vaccine-specific immunity, there is a need for rigorous longitudinal mechanistic and efficacy studies in paediatrics for different vaccines.

Technology and artificial intelligence. The use of artificial intelligence (AI), a specialised branch of computer science that deals with the ability of computers to perceive their environment and make decisions to maximise the chances of success of an event or goal, in African health systems is still limited but presents great potential. AI solutions can be used for decision support/validation, multiple-disease screening and diagnosis, including the use of genomic data and treatment optimisation within resource-constrained environments. A recent report presented a solution that integrates and analyses data across various sources, including disease incidence for clinical and operational decision support at the district level in Sierra Leone [111]. Disease screening, which involves reading images, can be fully or partially automated

using advances in computer vision and AI algorithms, coupled with the widespread availability of cell phones in Africa to enhance human expert capacity [112]. Network analyses can be used to establish links between diseases, ultimately informing treatment plans at the individual and population level [113]. Improving the rate of uptake and integrating AI platforms to electronic health records will improve individual healthcare as well as strengthen the African health system [114].

Mobile devices have become very popular in Africa and present great potential for improved healthcare delivery. An example is the mTRAC mobile health system being used in Uganda to report available stocks of medicines and the mHealth in Kenya to better understand the supply chain of medicines [115]. These will ensure that medicines reach patients who need it the most.

Where to go from here

Overall, we are in an era in which there is long-term survival and management of chronic conditions. Some health systems have resources for chronic long-term care and support groups are available in some countries to empower and promote healthy lifestyles for patients living with such conditions; an example is Diabetes South Africa. The challenge is thus to equip current health systems to shift from episodic interventions for acute care and be resourced for chronic care and to make existing support groups and systems readily available to the poorest and illiterate groups of affected patients.

Adequate training should be available to next generation scientists and health workers to build local health, research, and development capabilities. Ongoing programmes include the Human Health and Heredity in Africa (H3Africa), aimed at training local scientists from Africa to develop treatments for conditions including infectious (e.g., tuberculosis and malaria) and noninfectious diseases (e.g., cardiovascular disease) [115]. Guidelines, drugs, and appropriate monitoring equipment also need to be in place, accessible but affordable to all and tailored to different levels of care [51].

The role of education through interactive media and mobile devices cannot be overemphasised. There have been calls for funding agencies, the media, and health institutions in Africa to be partisan in health knowledge generation and application beyond publication in scientific journals [116]. Development partners have promoted innovative ways of delivering HIV and reproductive health education to young people; for example, the MTV Shuga show (http://www.mtvshuga.com/). The use of mobile phones is on the rise in Africa and present enormous potential for mobile health beyond direct patient care [117]. The mobile messaging platform WhatsApp has been demonstrated to be effective in enhancing the supervision of CHWs and creating innovative forms of community-based digitally supported professional development with minimal training [118]. This will go a long way to strengthen the formal healthcare system and enhance the role of CHWs in hard-to-reach areas. The photo sharing platform Instagram has been used by WHO, CDC, and others to broadcast public health messages for education and for sensitisation during public health crises [119].

A marked difference in healthcare can also be achieved through public–private partnerships. Ongoing initiatives like the Foundation for Chronic Disease Management (FCDM) by Novartis, in collaboration with International Business Machines (IBM) and Vodacom, is doing a great job by linking public health workers to those in the private sector to deliver quality but affordable healthcare to homes [115].

By conducting more target-oriented research in multiple disease systems, more realistic interventions will be achieved quickly for coinfections and comorbidities. To do this, researchers must shift toward collaborative and multidisciplinary studies, which can aid in

understanding disease interactions and their impact on overall health. Funders should be more willing to support such studies and be willing to fund riskier innovative research programmes with potential to benefit the health and wellbeing of millions of people. In addition, governing bodies and policy makers should be willing to incorporate findings from such studies and to prioritize both infectious and noninfectious diseases management.

Key learning points

- There is an epidemiological transition in sub-Saharan Africa (SSA), with the insurgence of coinfection and comorbidities from both infectious/neglected tropical diseases (NTDs) and noninfectious diseases.
- Health systems in SSA are ill equipped to deal with this in terms of diagnosis, intervention, and long-term care.
- Basic scientific research in SSA must be target oriented, collaborative, and on multiple disease systems (i.e., horizontal approach) to enhance our understanding of disease interactions and their impact on overall health and to improve implementation.
- Current success stories or interventions in the management of infectious and NTDs in SSA can be leveraged for noninfectious diseases, addressing coinfections and comorbidities.

Top five papers

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However, O_2 at levels above physiologically relevant concentrations can be toxic, causing elevation of oxidative stress in cells [11]. *Pf* evolved to grow and proliferate in RBCs, and almost immediately after RBC invasion the parasites start to feed on haemoglobin, resulting in the generation of free radicals. In order to counteract the increased stress response, the parasite must initiate a complex and well-orchestrated antioxidant response and maintain equilibrium throughout the complete asexual life cycle [12]. Trophozoite and schizont forms, in which high levels of haemoglobin digestion occur, are constantly required to balance what may be incremental increases in stress levels throughout development – perhaps explaining why trophozoites are more sensitive to drugs generating oxidative stress as part of their mode of action in comparison to young ring forms.

Thus, O_2 levels greater than those which are physiologically relevant to the parasite may potentially be detrimental, causing elevation in oxidative stress, specifically regarding the trophozoite forms. Trophozoites are known to adhere to endothelial cells in various blood vessels, tissues, and organs, and in the case of *Pf,* spend the majority of their development within recognized areas of lower oxygen (1–5%) than in the lungs and arteries [7]. The phenomena of cytoadherence and sequestration are well established as mechanisms to evade the host immune response, and mechanical removal of the iRBCs by the spleen. However, another reason may be to enable this microaerophilic parasite to elude contact with higher O_2 levels present in other parts of the blood circulation, thus, continually protecting itself from adverse events.

It is our opinion that O_2 levels between 1 and 5% are the predominant physiological conditions for *Pf in vivo,* and that O_2 levels provided in standard CO_2 balanced incubators (18.6%) do not correlate to *in vivo* conditions for trophozoite stages of the parasite and are therefore likely detrimental to parasite growth *in vitro*. Some strains of *Pf* are capable of proliferating within higher O_2 levels *in vitro* (18.6%), but the consequences to parasite transcriptomics, proteomics, genomics, and metabolomics data etc. should be considered when performing *in vitro* cultivation within these conditions.

Disclaimer Statement

Neither of the authors has any conflict of interest to declare.

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Science & Society

Universal Health Coverage in Africa: Coinfections and Comorbidities

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At the 67th session of the World Health Organization (WHO) Regional Committee meeting in August 2017, African health ministers adopted a range of transformational actions intended to strengthen health systems in countries, leading to Universal Health Coverage (UHC). A critical challenge for UHC is the existence of coinfections and noncommunicable diseases (NCDs), characterised by comorbidities.

African governments and international development partners have invested significant infrastructure, personnel, and health-delivery systems for infectious disease management and control strategies. To successfully deliver UHC, particularly related to NCDs, African health systems and research agenda need to leverage infectious disease expertise and resources.

Sub-Saharan Africa (SSA) is at a heightened risk of comorbidities from infectious diseases and a rising prevalence of NCDs. The growing burden of NCDs, for example, hypertension, stroke, cancer, and diabetes, may be linked directly

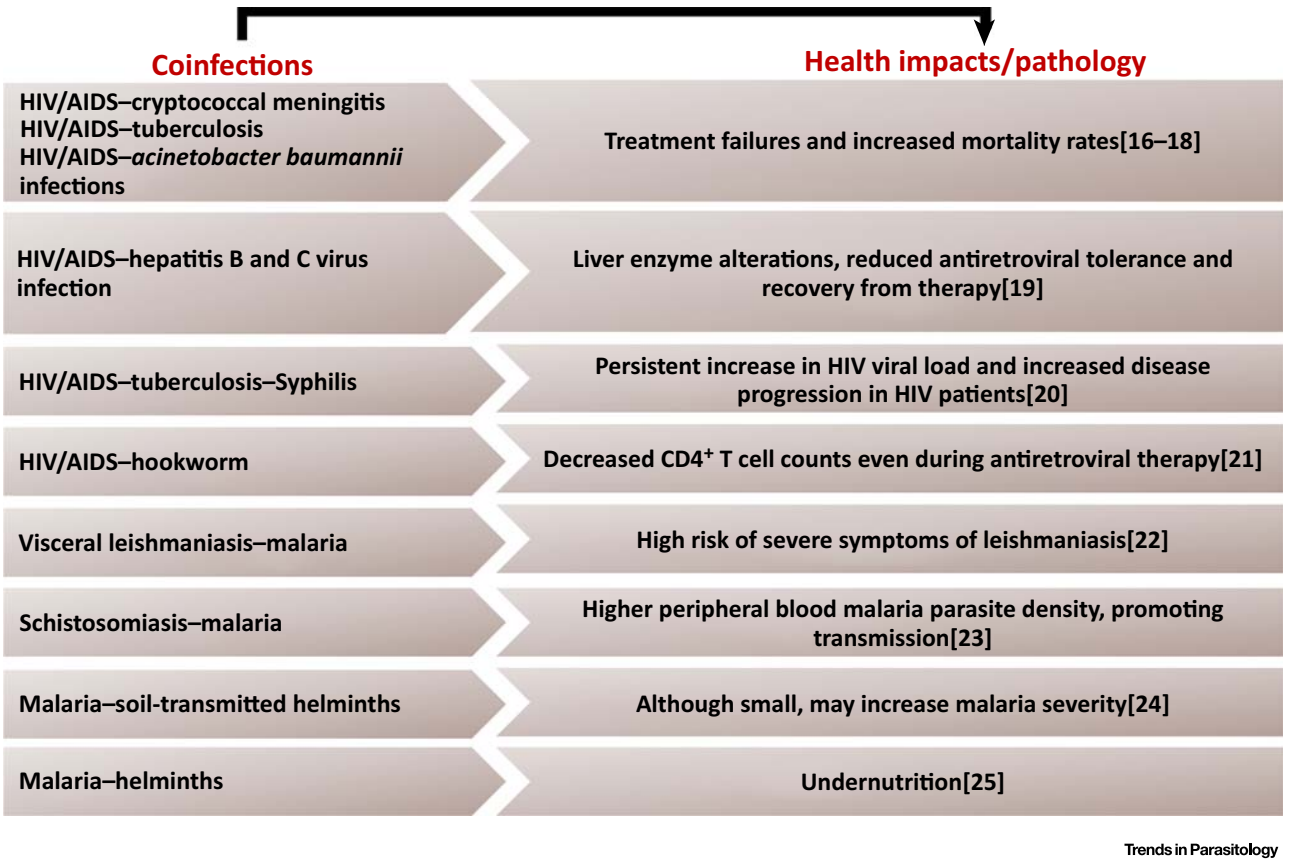

Figure 1. Detrimental Effects and Health Impacts of Coinfections as Described in Sub-Saharan Africa [16–25].

and indirectly to, and further exacerbated by, infectious diseases, including neglected tropical diseases (NTDs), which are prevalent in the region. This epidemiological transition is a 'time bomb' for Africa; the region is expected to record the world's largest increase in NCD deaths by 2030 [1].

Coinfections from various combinations of infectious diseases are common in SSA. Common amongst them are HIV, tuberculosis (TB), and malaria. The outcome and health impacts of such coinfections are summarised in Figure 1. It is becoming apparent that coinfections in humans can influence population-level disease and mortality patterns. This impacts on the nature and delivery of targeted interventions. Furthermore, infectious diseases have been linked to the emergence of NCDs, and sometimes complicate or increase the risk of acquiring NCDs (Figure 2). The success and effectiveness of control and research programmes focusing on infectious diseases, provide opportunities for integrating and developing interventions for coinfections and comorbidities.

UHC for Africa will mean that everyone can access promotive, preventive, curative, rehabilitative, and palliative health services. This will need to be of appropriate quality to be effective, while ensuring affordability[i]. African health systems are built on a model for controlling infectious diseases, providing more of episodic and acute care, with exceptions for HIV management. Given this foundation, we pose two questions: (i) what lessons can we learn from infectious disease-control programmes in Africa, and (ii) how can we leverage these lessons, expertise, and resources to deliver UHC for comorbidities? It is thus informative to review some successful infectious disease-control programmes.

HIV and TB

Due to increased access to antiretroviral therapy and preventive measures, including condoms and public health campaigns, AIDS-related deaths are declining in SSA; in some parts, by up to 50%[ii]. However, this progress means that HIV is now a chronic infection requiring long-term management, with a substantial increase in the risk of coinfections and NCDs, including diabetes. TB, for example, accounts for about a third of AIDS-related deaths worldwide, with the biggest impact on Africa. Programmes

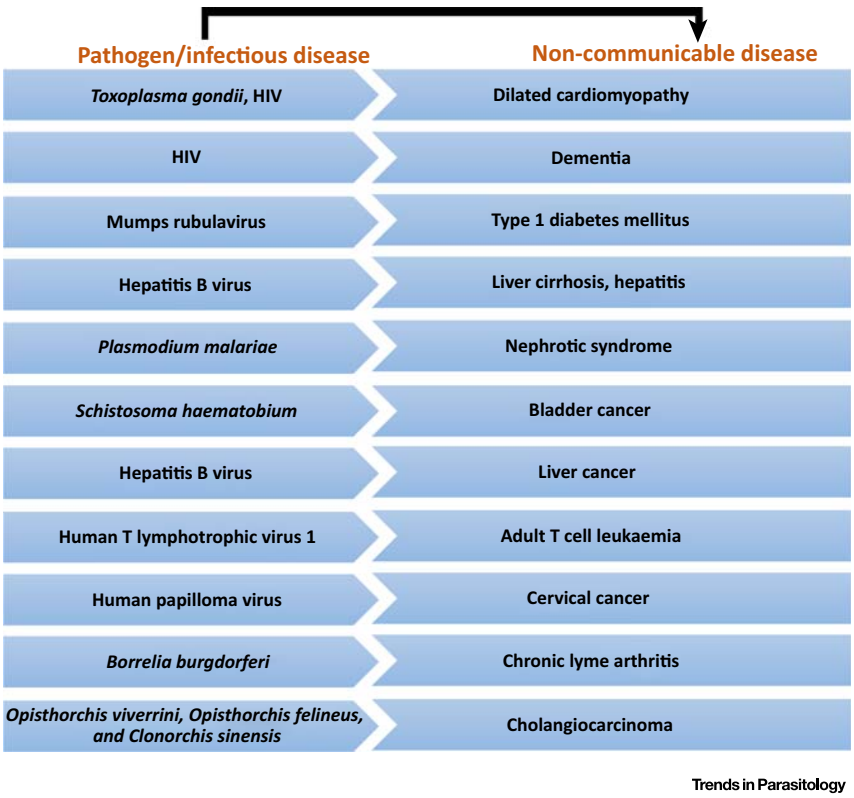

Figure 2. Noncommunicable Diseases and Their Infectious Disease Links or Causes [26,27].

such as the integrated HIV/TB therapy are thus crucial for all people living with HIV (PLWH). The success of this model is in the feasibility to setup and maintain. Furthermore, the clincal care for both infections has been intergrated to be available from a single provider. This model also promotes the training of health workers.

Such integrated care can help to reduce loss to follow-up during patient referrals, reduce travel costs in and out of hospitals [2], and ensure judicious use of limited resources. In many parts of SSA there is a geographic overlap between HIV/AIDS, TB, and malaria, and in such places most individuals have multiple worm infections [3]. HIV/TB control measures can thus be scaled up to include NTD and NCD control. An example is the Tanzania Essential Health Interventions Project, which is serving as a framework for the control of other NTDs. In such projects, routine checks for possible coinfections, hypertension, and diabetes are incorporated into HIV/TB programmes; thus, PLWH will get a holistic health evaluation, ensuring better clinical outcomes.

Mass Drug Administration (MDA)

MDA involves the periodic treatment of a specific population, irrespective of infection status, where treatment is cheaper than diagnosis. The majority of infections from soil-transmitted helminths (STHs), lymphatic filariasis, and schistosomiasis are treated with MDAs, with over 700 million treatments given annually. MDAs, 'a global best buy', are a success because they are cheap, effective, have wide coverage, reduce morbidity and infection burden, and can reduce transmission and enhance global health [4]. Lessons from the success of MDAs include strong community-wide sensitization, distribution in schools/clinics to improve access, and close supervision of treatment by health workers, all tailored to infection patterns of specific populations.

Following the geographical overlap between the major NTDs and NCDs alike, the already-working model of MDAs can be leveraged for the simultaneous control of coinfections and comorbidities. For example, in some programmes, routine praziquantel treatments for schistosomiasis are supplemented with the administration of albendazole (for STHs) and routine screening of malaria. Although time consuming, the screening for NCDs, including body mass index and blood glucose measurements, can be incorporated into MDA programmes as they tend to attract multiple generations within a family. An example is the inclusion of vitamin A supplementation in Zimbabwe's 2017 round of helminth MDA and the WHO's MDA of antibiotics as a strategy to clear trachoma infection and reduce transmission in endemic communities. A recent trial on using broad-spectrum antibiotics to promote child health showed that an MDA with azithromycin reduced child mortality. Caution is however suggested with this approach due to the potential of facilitating the spread of antibiotic resistance [5].

Expanded Programme on Immunisation (EPI) and Global Polio Eradication Initiative (GPEI)

The EPI, first introduced in 1976, was to make vaccines against the six main childhood killer diseases available to every child, as part of achieving health for all by the year 2000. Following the eradication of smallpox, the GPEI was established as part of national immunisation days, which have seen a reduction in polio cases by over 99%[iii]. As of 2016, only Afghanistan, Nigeria, and Pakistan remain with ongoing transmission[iv]. The GPEI's main highlights for success are the influence of organisational partnership,

national leadership, and strong community engagement. This shows that well-planned interventions can reach the most remote, conflict-affected, or poorest areas.

The successes and infrastructure of immunisation programmes can be leveraged for the control of infectious diseases and NCDs by linking them with other interventions, ensuring that they are well coordinated. Due its simplicity, many countries have also linked deworming with routine vaccination schedules to improve coverage. Routine screening of other infections, including HIV and malaria, provide opportunities for early infant diagnosis and overall child health. Immunisation has also provided the avenue for the distribution of impregnated bed nets, as well as access to community members for educational campaigns on behavioural change for infectious diseases and vector control. Highlighting the link between infectious diseases and NCDs, EPIs with concomitant screening and treatment of other infectious diseases have the potential to protect the larger population from both infectious diseases and NCDs.

Research Questions

To leverage these success stories to deliver UHC for coinfections and comorbidities in Africa, knowledge gaps in key areas need to be addressed. Below, we explore these areas and the importance of leveraging lessons from infectious disease control.

Diagnosis

In Africa, most health systems lack basic diagnostic tests to explain clinical symptoms, and there are challenges with quality control, reproducibility, and national laboratory guidelines. A significant number of diagnostic tests currently available have been developed and validated in the West, without appropriate validation in Africa. For example, in a Zimbabwean population with a high prevalence of helminths, there is a falsely low prevalence of allergies, as routine allergy diagnosis is impaired by IgE antibodies to the carbohydrate epitope galactose-α-Gal, induced by the parasite infection [6]. The sensitivity of conventional egg-detection methods for helminth diagnosis is reduced in light/prepatent infections, underestimating infection rates [7]. Therefore, tests must be sensitive, specific, user-friendly, and applicable in resource-limited settings. This is vital for accurately quantifying disease burden and epidemiology, informing policy, and creating health systems geared towards the needs of Africa.

Appropriate diagnosis will not only help to identify those who need treatment, but also prevent over- or under-treatment, which lead to drug inefficacies; for example, resistance to treatment of malaria with chloroquine. New technology must be informed by social and cultural contexts which may hinder the acceptance and widespread use of diagnostics. A working example is where health experts find and track outbreaks, routine diseases, and genetic risk of diseases, by combining the locally accepted verbal autopsies with minimally invasive autopsy, in conjunction with cheap needle sampling methods [8]. Diagnosis informs contextualised interventions, and necessitates customisation for specific populations; for example, developing specific reference ranges for the African population. For example, in HIV management, initiation of antiretroviral therapy is informed by CD4 cell counts and inappropriate standard values will mean inappropriate interventions [9]. A gap currently exists in multiplex diagnostic platforms that can be used at point of care. It is noteworthy that several disease biomarkers are detected in urine and sera, so developing platforms that can detect pathogen and physiological markers concurrently in a single sample will significantly strengthen African health systems.

Interventions: Integrated Approach

Interventions must be targeted, informed by knowing the desired outcome and what tools are required/available to achieve this. The vertical approach of most interventions targeted against specific diseases should be modified into a horizontal approach to benefit other conditions. In the Solomon Islands, ivermectin has been used in scabies control programmes to reduce secondary streptococcal skin infections and renal disease [10]. Solutions targeting multiple conditions are receiving increased attention. These include water, sanitation, and hygiene strategies targeting better growth outcomes in children [11], and integrated interventions delivered concurrently in health systems, homes, and communities to improve breastfeeding practices for overall child health [12]. More studies demonstrating the positive health impact of integrated approaches are required, particularly where health budgets are already stretched.

Drugs and Drug–Drug Interactions

In the face of coinfections, multiple drugs for multiple conditions are inevitable, necessitating the need to consider drug–drug interactions. In patients coinfected with HIV and TB, a lopinavir/ritonavir combination in HIV therapy has been found to significantly increase the exposure and toxicity of bedaquiline, used during TB treatment [13]. In multiparasitic interventions, coadministration of praziquantel along with albendazole may affect the total exposure of albendazole [14]. Very few drugs used in Africa have been evaluated in African populations in terms of drug–drug interaction and pharmacogenetics. The advent of African-specific molecular tools, for example, an African-specific genome-wide association studies (GWAS) array,

will facilitate the study of genetic factors involved in disease susceptibility and drug responses[v]. Phase IV clinical trials will inform African health systems of appropriate drug coprescription.

Concluding Remarks

In the face of this epidemiological transition, a vehicle for delivering UHC in Africa will be the adaptation of an alternative, flexible, and horizontal disease network approach to diagnosis and management. Current successful intervention programs for infectious diseases that require community mobilisation and engagement, behavioural change, long-term adherence to treatment and monitoring have already laid the foundation for coinfection/comorbidity management in resource-poor settings. The increased involvement of community-based health workers, new technology at primary health centres coupled with the widespread availability of cell phones and social media in Africa, must be harnessed for disease screening, training, and supervision. Artificial intelligence technology, already being used for some infectious disease programs in Africa, will enhance human expertise/capacity in the diagnosis and management of coinfections and comorbidity [15].

Resources

[i]www.who.int/health_financing/universal_coverage_definition/en/

[ii] www.unaids.org/sites/default/files/media_asset/20170720_Data_book_2017_en.pdf

[iii]www.who.int/en/news-room/fact-sheets/detail/poliomyelitis

[iv]www.unicef.org/partners/files/Partnership_profile_2012_Polio_revised.pdf

[v]www.illumina.com/company/news-center/feature-articles/h3africa-consortium-array-available-soon-.html

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COMMUNICATIONS BIOLOGY

ARTICLE

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The gut microbiome but not the resistome is associated with urogenital schistosomiasis in preschool-aged children

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Helminth parasites have been shown to have systemic effects in the host. Using shotgun metagenomic sequencing, we characterise the gut microbiome and resistome of 113 Zimbabwean preschool-aged children (1–5 years). We test the hypothesis that infection with the human helminth parasite, *Schistosoma haematobium*, is associated with changes in gut microbial and antimicrobial resistance gene abundance/diversity. Here, we show that bacteria phyla *Bacteroidetes*, *Firmicutes, Proteobacteria*, and fungi phyla *Ascomycota, Microsporidia, Zoopagomycota* dominate the microbiome. The abundance of *Proteobacteria*, *Ascomycota*, and *Basidiomycota* differ between schistosome-infected versus uninfected children. Specifically, infection is associated with increases in *Pseudomonas, Stenotrophomonas, Derxia, Thalassospira*, *Aspergillus, Tricholoma*, and *Periglandula*, with a decrease in *Azospirillum*. We find 262 AMR genes, from 12 functional drug classes, but no association with individual-specific data. To our knowledge, we describe a novel metagenomic dataset of Zimbabwean preschool-aged children, indicating an association between urogenital schistosome infection and changes in the gut microbiome.

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The human gut comprises a diverse ecosystem of microbes, predominantly bacteria, in addition to viruses, fungi and other eukaryotes[1]. Evidence shows that humans rely on the symbiotic relationship with the resident microbial taxa present in humans (microbiota) for extracting essential nutrients from food, as a first line of protection from pathogens, and as a mechanism for shaping the immune system[2]. Shotgun metagenomic sequencing has allowed characterisation of the microbiome (the assembly of genomes of the microbiota) among different human populations, showing considerable heterogeneity[2,3]. Populations in Africa have been underrepresented in such studies, with a major focus on Western populations[4]. Other studies have included diverse but older populations, not allowing the factors inherent to African childhood to be fully disentangled[5–8]. Given the potential window of opportunity for influencing health through the microbiome in infants and young children[9], research focus on this age group is important. Findings from consortiums including the Human Heredity and Health in Africa (H3 Africa) and the HapMap Project will be invaluable for informing nutraceuticals in Africa[10,11].

The composition of the gut microbiome is influenced by age[8,12], diet and geography[5,13,14], host genotype[15], exposure to maternal microbiota[16], as well as environmental factors[17] including the role of protozoal and helminth parasites[18]. In Africa, children are exposed to several acute and chronic parasitic infections that can impact children's growth and development[19]. In particular, helminth parasites (as shown for schistosome worms) can be contracted by children as young as 6-month-old or less[20,21], and these can persist into the second decade of life where they modulate the immune system as well as cause morbidity and pathology[22]. In very young children, the gut microbial population continues to evolve until about age 3–5 years[8,12], thus it is important to establish how external factors, especially infections, that young children are exposed to, influence the microbiome.

Schistosomiasis is a disease caused by infection with trematodes of the genus *Schistosoma*—the predominant human species found in Africa being the urogenital (*S. haematobium)* and intestinal (*S. mansoni*) forms[23]. Pathology from the disease is mostly from immunological reactions to trapped eggs attempting to migrate through to the bladder or intestinal lumen, depending on the species involved. The infection causes immunomodulatory effects which help to promote both parasite and host survival[24,25], and in preschool-aged children, consequences can extend to malnutrition, poor growth and cognition, reduced vaccine efficacy, and altered prognosis of co-infections[26,27]. Treatment for schistosomiasis is through administration of the antihelminthic drug, praziquantel, which is effective against all schistosome species[28].

A number of experimental and human studies, including our own, have examined the association between helminth infections and the structure and composition of the gut microbiome[29–35]. It has been suggested that the immunomodulatory effects of schistosome infection can extend to the gut microbiota through direct intestinal or systemic interactions[18]. Work in experimental models shows that depletion of the gut bacteria is associated with reduced *S. mansoni* egg excretion, gut pathology and inflammation[32]. Recently, fluctuations in the composition of the gut microbiota of mice infected with *S. mansoni*, before and after intestinal damage from egg transmission was shown[36]. This is consistent with a role of the mammalian gut microbiota in the pathogenesis of schistosome infection. However, unlike *S. mansoni* (intestinal form) that inhabits the same environment as the gut microbiota, *S. haematobium* predominantly resides in the venous plexus of the bladder (although occasionally in the mesenteric circulation[37]), and thus presents a need to study the indirect systemic impacts of infection on the gut microbiota—a more likely interaction. Correlations between the gut microbiome and systemic diseases such as rheumatoid arthritis suggest the importance of such systemic interactions[38]. Phenotypic and mechanistic studies on systemic interactions between helminths and the microbiome in natural human infections are still in their infancy, and more studies are needed. In a previous study, we found differences in the gut microbiome between *S. haematobium* infected versus uninfected children, aged up to 13 years old[35]. This was supported by Schneeberger et al.[34], suggesting that genetic and environmental factors may play an additional role. Recently, a study conducted among older children (11–15 years) in Nigeria showed that urogenital schistosomiasis is associated with disruptions in the gut microbiome, suggesting that this may be a further consequence of schistosome infection[39]. However, substantial knowledge gaps on the interaction between the gut microbiome and *Schistosoma* infection in preschool-aged children still exist. The biggest challenges are demonstrating causation and elucidating mechanistic pathways for any existing interactions.

In addition to the schistosome–microbiota interactions, other interactions relevant to the health of the host have also been reported to occur within the gut ecosystem. One such example is the ability of *Salmonella* to persist in the body by attaching to intestinal schistosomes, evading repeated antibiotic treatments, increasing the *Salmonella* population and eventually, potential antibiotic resistance[40,41]. Furthermore, the microbiome is a reservoir for antimicrobial resistance (AMR) genes (resistome)[42,43], and provides an ideal environment for AMR gene exchange among the "resident" and transitory bacterial population[44]. Such interactions are likely to impact the structure and diversity of the resident microbial population, as well as the overall AMR gene composition. We therefore investigated the structure of AMR genes to bacteria and determined if this was associated with any host-related factors including socio-demography, antibiotic use, current schistosome infection, as well as feeding, growth and nutritional indices. AMR remains one of the largest threats to human health, with numerous calls for antibiotic resistance stewardship worldwide[42,45,46]. However, this population of African preschool-aged children is understudied, and almost all AMR gene studies are conducted in industrialised settings[43,47–50]. Such settings contrast with low-and middle-income countries in terms of access to safe water and sanitation, and access to antibiotics, with or without prescriptions[51].

Within the framework of a larger paediatric schistosomiasis study in Zimbabwe, the present study focuses on shotgun metagenomic sequencing of stool samples from preschool-aged children, aged 1–5 years old. To add to the repository of information on the gut microbiome and AMR studies in this young population, we characterise the structure and diversity of the gut microbiome (to include the fungi repertoire) and resistome. We apply these data to test the hypothesis that *S. haematobium* infection is associated with alterations in the gut microbial and AMR gene abundance and diversity. We find that the microbiome but not the resistome is associated with *S. haematobium* infection, independent of age, sex and village.

Results

Population characteristics. Of the 113 participants included in the study, the mean age was 3.7 ± 1.1 years, of which 56 were females (49.6%). Sixty-eight (60.2%) and 45 (39.8%) children were from Chihuri and Mupfure villages, respectively. Antimicrobial use data showed that 58 (51.3%) participants had received antibiotics [amoxicillin (31), co-trimoxazole (27), both (9)], while 18 (15.9%) had not; no information was obtained for the remaining 37 (32.7%) participants. Previous history of praziquantel treatment (for schistosome infection) was reported

among 29/105 (27.6%) children. *S. haematobium* infection prevalence was 15.9% (18/113), with mean infection intensity of 1.79 eggs/10 ml urine (SEM = 0.76; range = 0–74).

We gathered data on the history of feeding habits and nutritional status of the children. The majority (83.6%) were breastfed, with duration ranging from 2 to 48 months (median = 18 months IQR: 17–20). Children were introduced to solid foods between 1 and 24 months after birth. Diet comprised mainly of traditional maize flour porridges (97%; 96/103), the commercial Cerelac® porridge (1.9%; 2/103), and potatoes (1%; 1/103). Anthropometric measures, adjusted for age, were used to assess nutritional status[27]. Based on the weight-for-height *Z* scores (WHZ), 3.7% (4/107) of individuals were malnourished and 14.7% (16/109) were stunted, based on the height-for-age *Z* scores (HAZ)[52].

Taxonomic composition of the microbiome. The number of classified read pairs per sample ranged from 3,994,704 to 13,164,482. An average 45.1% of read pairs were mapped to specific reference sequences in the genomic database; this is similar to other studies with the proportion of unmapped reads ranging from 42% to 68%[53–55]. At any taxonomic level, a putative taxonomic classification could not be assigned to at least 33% of the mapped read pairs and were thus classified as "Unknown".

In the 113 stool samples, 845 bacteria genera (from 20 unique phyla) and 228 fungi genera (from six unique phyla) were detected. As shown in Fig. 1, the most abundant bacteria phyla in decreasing order were *Bacteroidetes* (genera: *Prevotella, Bacteroides, Alistipes*), *Firmicutes* (genera: *Eubacterium, Faecalibacterium, Clostridium, Roseburia*), and *Proteobacteria* (genus: *Succinatimonas*). The most abundant fungi phyla were *Ascomycota* (genera: *Protomyces, Aspergillus, Taphrina, Saccharomyces, Candida, Nakaseomyces*), *Microsporidia* (genus: *Enterocytozoon*), and *Zoopagomycota* (genus: *Entomophthora*) [Fig. 2]. These phyla dominated the microbiome and were present in all samples.

Variation in the microbiome and association with sample metadata. Principal component analysis (PCA) was used to initially examine variability and patterns in the data set across the first two principal components. At the phylum level, PCA explained 62% and 42.0% of the total variation in fungi and bacteria, respectively. At the genus level, however, PCA explained 34% and 16% of the total variation in fungi and bacteria respectively. The model showed homogeneity in components with no distinct clustering according to metadata and may reflect a high diversity in the cohort. PCA plots and cluster dendrograms for bacteria and fungi content per sample is shown in Supplementary Figs. 1–3.

Permutational multivariate analysis of variance (PERMANOVA) analysis showed a significant effect of age (false discovery rate (FDR) = 0.024) and village (FDR = 0.039) [details shown in Supplementary Fig. 4], schistosome infection status (FDR = 0.039) and schistosome infection intensity (FDR = 0.012) on bacteria genera, across samples. There was also a significant effect of schistosome infection status (FDR = 0.006) and schistosome infection intensity (FDR = 0.006) on fungi genera, across the samples. For both bacteria and fungi genera, no such effects were found for sex, nutritional and growth variables, feeding, previous

Fig. 1 Overview of bacterial microbiota abundance and composition. From read mapping to the genomic database, abundance was calculated for each microbial taxa across all samples. Stacked bar charts show the most abundant bacteria (**a**) phyla and (**b**) genera per sample, proportional to the total microbiota within each sample ($n = 113$ biologically independent samples). Charts were generated using normalised, zero-corrected abundance matrices. "Unknown" represents abundance data for which a putative taxonomic classification could not be assigned. "-Others" represents abundance data for all other taxa in the abundance data set.

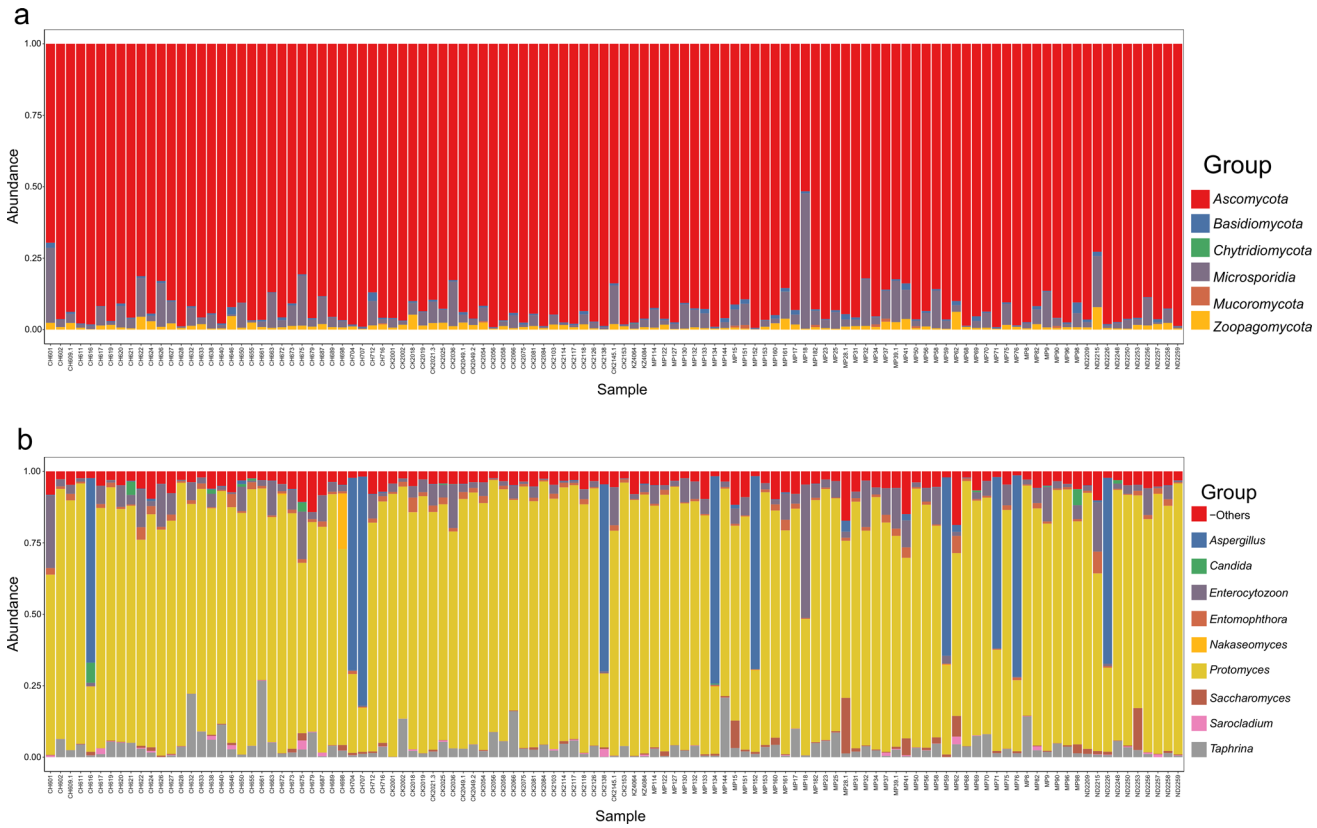

Fig. 2 Overview of fungal microbiota abundance and composition. From read mapping to the genomic database, abundance was calculated for each microbial taxa across all samples. Stacked bar charts show the most abundant fungi (**a**) phyla and (**b**) genera per sample, proportional to the total microbiota within each sample ($n = 113$ biologically independent samples). Charts were generated using normalised, zero-corrected abundance matrices. "-Others" represents abundance data for all other taxa in the abundance data set.

praziquantel treatment, and antibiotic use (FDR > 0.05). Summary output from the analysis is shown in Table 1.

Different genera by schistosome infection status and intensity. We investigated further, via analysis of composition of microbiomes (ANCOM), how specific bacteria and fungi genera were associated with *S. haematobium* infection, while controlling for age, sex and village, followed by evaluation for association with infection intensity. In total, eight genera were identified, five from bacteria (*Pseudomonas*: $W = 347$, *Azospirillum*: $W = 346$, *Stenotrophomonas*: $W = 292$, *Derxia*: $W = 288$, and *Thalassospira*: $W = 292$) and three from fungi (*Aspergillus*: $W = 75$, *Tricholoma*: $W = 73$ and *Periglandula:* $W = 70$). The magnitude of these changes were shown by plotting the abundance of each sample to highlight differences between groups. In schistosome-positive children, the abundance of all but *Azospirillum* was higher (Fig. 3a–e). This observation was consistent with infection intensity [*Pseudomonas* ($r = 0.3$; $p = 0.001$), *Stenotrophomonas* ($r = 0.4$; $p < 0.001$), *Derxia* ($r = 0.6$; $p < 0.001$), *Thalassospira* ($r = 0.6$; $p < 0.001$) and *Azospirillum* ($r = -0.4$; $p < 0.001$)] as shown in Fig. 3f–j. Likewise, the abundance of *Aspergillus, Tricholoma*, and *Periglandula* was higher in schistosome-positive children (Fig. 4a–c) and was consistent with infection intensity as shown in Fig. 4d–f [*Aspergillus* ($r = 0.5$; $p < 0.001$), *Tricholoma* ($r = 0.5$; $p < 0.001$), and *Periglandula* ($r = 0.4$; $p < 0.001$)].

AMR gene characterisation. An average 0.06% of read pairs were mapped to AMR genes in the ResFinder database. We found evidence of 262 AMR genes, belonging to 12 functional drug class levels. AMR genes belonging to tetracycline was the most common, followed by beta-lactam, macrolide, sulfonamide and nitroimidazole. Of these, the most abundant genes were *cfxA6*, followed by *tet(Q), tet(W), sul2, erm(F)* and *nimE* (Fig. 5).

Variation in the resistome and association with sample metadata. PCA was used to initially examine variability and to identify clustering according to individual metadata. The model for the first two components explained 18.0% and 48.0% of the total variability in AMR genes and drug classes respectively. Similarly, there was no clustering according to individual metadata, reflecting high cohort diversity and the role of other factors in influencing the resistome. PCA plots and cluster dendrograms of AMR genes and their drug classes per sample is shown in Supplementary Figs. 5–6.

PERMANOVA analysis did not show any significant association of AMR genes with age, village, sex, feeding, malnutrition, stunting, *S. haematobium* infection, previous praziquantel treatment and antibiotic use on AMR genes. Model summaries of sample metadata and association with AMR genes is shown in Supplementary Data 2.

Discussion

Using shotgun metagenomic sequencing, we characterised the structure and composition of the human gut microbiome and resistome in this Zimbabwean preschool population (≤5 years old). Age[8,12], dietary and environmental patterns[5,13,17], ethnicity, and geography[14,56] have a substantial impact on the taxonomic composition of the microbiome. *Prevotella* and *Candida*[57,58] have been associated with carbohydrate-rich diets, and *Bacteroides* with protein-rich diets[57]. This is a reflection of the dietary lifestyle

Table 1 Model summaries of sample metadata and association with the gut microbiome.

Variable	*n*	Bacteria				Fungi			
		p value	SSExplain	SSTotal	FDR	*p* value	SSExplain	SSTotal	FDR
Age (years)	113	0.004	1344.6	82733.4	0.024	0.082	128.6	9489.2	0.197
Sex	113	0.172	878.1	83200.0	0.258	0.439	82.8	9534.9	0.671
Village	113	0.012	1254.0	82824.0	0.039	0.060	140.4	9477.4	0.180
S.h. infection status (pos/neg)	113	0.013	1185.1	82892.9	0.039	0.001	339.0	9278.7	0.006
S.h. infection intensity	113	0.001	1514.5	82563.6	0.012	0.001	670.1	8947.7	0.006
Malnourished, yes/no (WHZ)	107	0.866	589.1	78498.2	0.913	0.830	59.5	9145.6	0.830
Stunted, yes/no (HAZ)	109	0.407	754.6	79751.0	0.542	0.611	71.5	9227.2	0.671
Months breastfed	90	0.082	954.9	64235.8	0.140	0.470	75.2	6985.0	0.671
Months solid food introduced	102	0.913	573.1	75583.0	0.913	0.615	73.5	8792.0	0.671
Previous praziquantel treatment	105	0.071	991.9	77387.4	0.140	0.233	101.6	8934.5	0.466
Amoxicillin (yes/no)	76	0.771	646.6	55603.4	0.913	0.531	78.4	6567.3	0.671
Co-trimoxazole (yes/no)	76	0.030	1083.6	55166.4	0.072	0.048	158.3	6487.4	0.180

The table represents PERMANOVA output for bacteria and fungi genera. Classification of nutritional status was based on a cut off <−2 *Z* scores[52]. Schistosome infection intensity was log transformed ($\log_{10}$ [egg count + 1]). *S.h. S. haematobium*, *WHA* weight-for height *Z* scores, *HAZ* height-for-age *Z* scores, *pos/neg* positive/negative, *p value* unadjusted *p* value, *FDR* adjusted *p* value (FDR-corrected), *SSExplain* explained sum of squares, *SSTotal* total sum of squares.

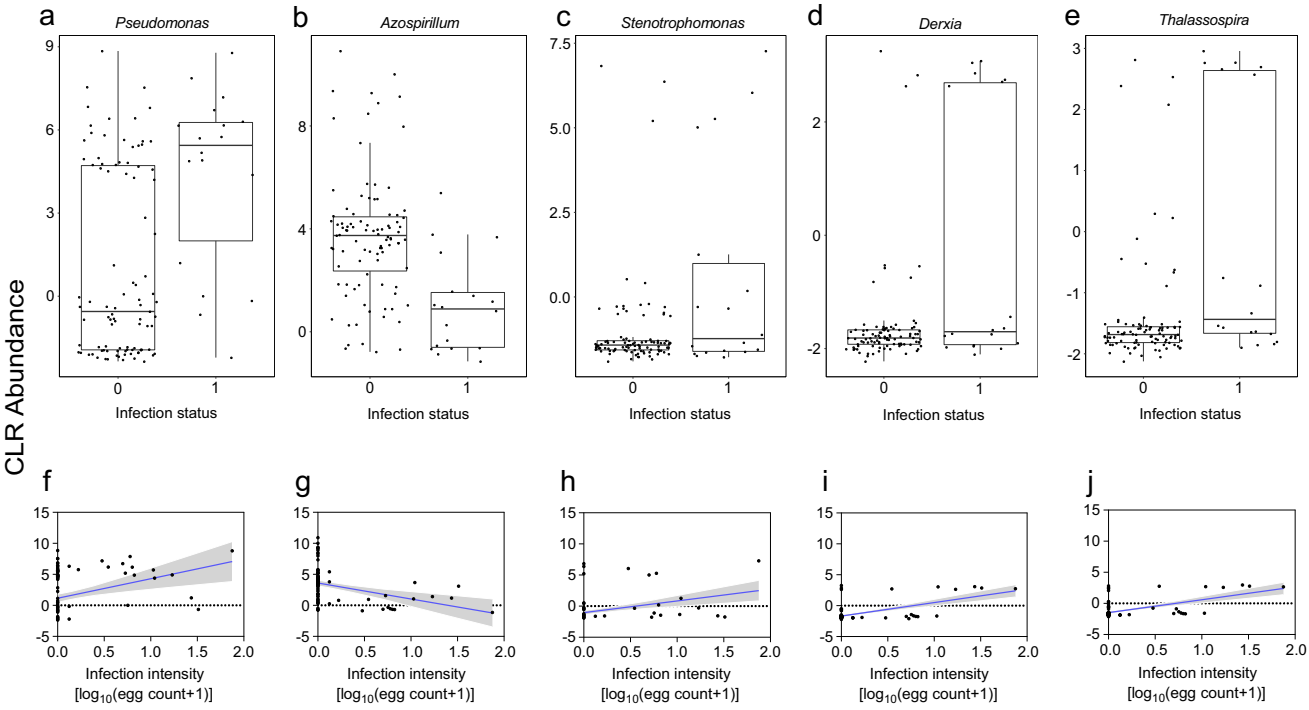

Fig. 3 Different bacteria genera between schistosome-infected children compared to uninfected children. (a–e) Box plots showing the abundance of specific bacteria genera, grouped by *S. haematobium* infection status. The horizontal box lines represent the first quartile, the median, and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range. (**f–j)** Scatter plots showing linear regression analysis of *S. haematobium* infection intensity and bacteria genera abundance. The clr-transformed abundance data were used for all plots. Infection status was coded as 0 and 1 for negative ($n = 95$) and positive ($n = 18$), respectively. *S. haematobium* infection intensity was log transformed [$\log_{10}$ (egg count + 1)]. Shaded areas indicate the 95% confidence intervals.

among populations in developing countries[5,17], including infants[59], and these genera were among the most abundant bacteria and fungi genera found in the current study population. Similar to our previous findings[35], we found age but not sex-related associations in bacteria genus diversity. Given that the population in the current study were ≤5 years old, this is consistent with the microbiome being more dynamic in the early years of life before stabilising to a more adult-like state[8,12].

Differences observed in the microbiome between developing and developed countries have been attributed to factors inherent to such developing areas[5], which may include the role of persistent prevalence of helminth infections, as reviewed by

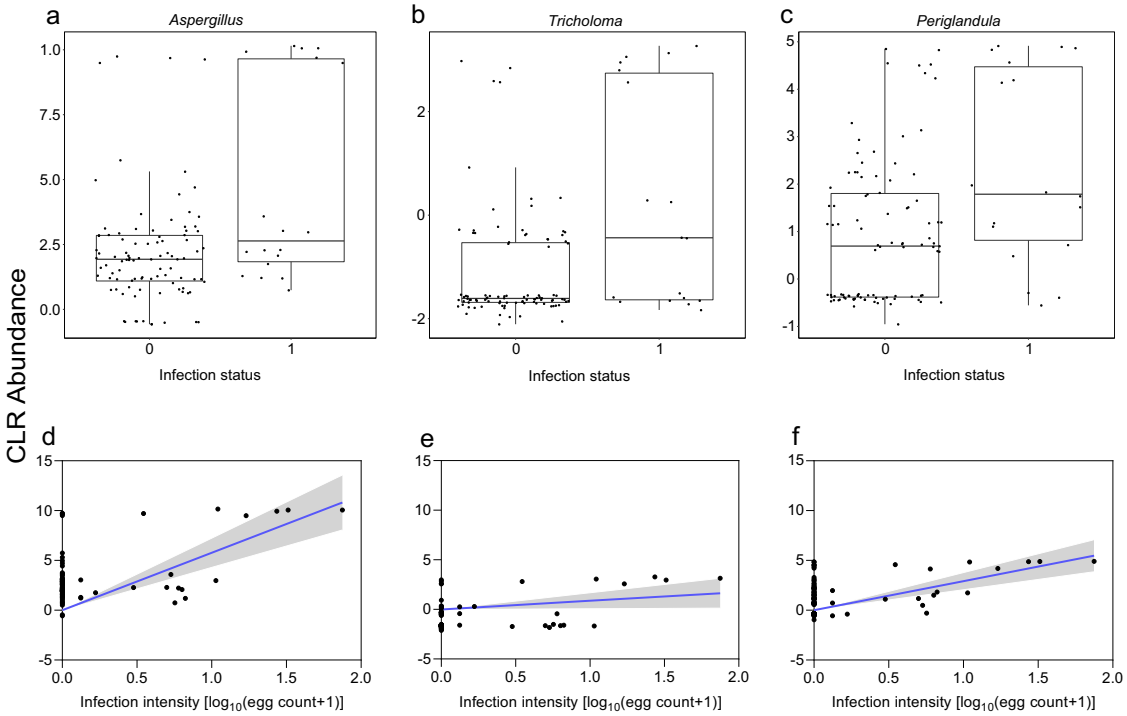

Fig. 4 Different fungi genera between schistosome-infected children compared to uninfected children. (a–c) Box plots showing the mean abundance of specific fungi genera, grouped by *S. haematobium* infection status. The horizontal box lines represent the first quartile, the median and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range. (**d–f)** Scatter plots showing linear regression analysis of *S. haematobium* infection intensity and fungi genera abundance. The clr-transformed abundance data were used for all plots. Infection status was coded as 0 and 1 for negative ($n = 95$) and positive ($n = 18$), respectively. *S. haematobium* infection intensity was log transformed [$\log_{10}$ (egg count + 1)]. Shaded areas indicate the 95% confidence intervals.

Mishra et al.[18]. Our findings are consistent with observations that schistosome infection is associated with alterations in the diversity and abundance of specific taxonomic groups in the microbiome[34,35]. In the aforementioned studies, which included preschool and school-aged children, 16S rRNA sequencing showed that *Prevotella* and *Proteobacteria* were more abundant in children infected with *S. haematobium*[35] and *S. mansoni*[34] respectively, when compared to uninfected children. To the best of our knowledge, the novelty of the current study is the fact that this population is much younger (≤5 years old), an age group whose gut microbiome structure is most likely still being established. Our study thus provides an important insight into helminth infection and its association with changes during the establishment of the gut microbiome in preschool-aged children in endemic areas. We also expand on this to include the fungal component of the microbiome. More relevant to our finding is that this association was independent of age, sex and village.

Phyla that clearly differentiated the microbiome of the schistosome-infected versus uninfected children were *Proteobacteria, Ascomycota* and *Basidiomycota*. These were among the top five most abundant bacteria or fungi phyla and were present in all samples, thus make a major contribution to the overall microbiome composition. *Proteobacteria* has been shown to be present in lower abundances in healthy individuals, and any increases in abundance of members of this phylum confirm dysbiosis and a link with increased disease risk, progression and burden[60]. Attempts have been made to expand the body of knowledge on the fungi repertoire and diversity in the human microbiome[61] and their association with infection and disease[62–64]. Studies have suggested that gut fungal populations directly or indirectly help to maintain healthy intestinal homoeostasis and that dysbiosis has immunological consequences[65]. Increases in specific fungi populations such as *Aspergillus* have been associated with increased eosinophil levels[66] and an exaggerated Th2 response[65], also characteristic of schistosome infection, which may explain our observation of the association of schistosome infection with specific fungal populations (*Aspergillus, Tricholoma* and *Periglandula)*. However, whether our observation was due to primary changes in the fungal population or were secondary to changes in the bacterial population is unclear. To the best of our knowledge, this is the first study to examine such an association and further studies into the role of fungal dysbiosis in schistosome infection are warranted.

Although we cannot infer causation, we are able to determine that for the genera differentiating the microbiome of the schistosome-infected versus uninfected children, there was a positive relationship between microbial abundance and schistosome infection intensity. Hence, it is possible that schistosome infection resulted in alterations in the gut microbiome. However as *S. haematobium* worms mostly reside in the pelvic venous plexus (although some have occasionally been detected in the intestine in Egyptian autopsies[37]), the effect of infection on the diversity of the microbiota is as suggested for intestinal helminths[33], but most likely through a more indirect or systemic route than through direct interactions[18]. Mishra et al.[18] have suggested that the immunomodulatory effects of helminths can extend to the gut microbiota through both direct intestinal interactions and systemic interactions. For example, by enhancing the mucosal barrier, tissue repair, production of antimicrobial peptides and reducing dissemination of microbiota to the spleen and liver[18], the upregulation of IL-22 during helminth infection may favour the abundance of specific microbial taxa[67].

We identified 262 AMR genes, most of which encoded for resistance to tetracycline, beta-lactam, macrolide and sulfonamide, posing risks to successful treatment of various conditions

Fig. 5 Overview of antimicrobial resistance (AMR) gene abundance and composition. From read mapping to the ResFinder database, AMR abundance was calculated for each reference gene across all samples. Stacked bar charts show the most abundant (**a**) AMR gene and (**b**) drug class per sample, proportional to the total AMR within each sample ($n = 113$ biologically independent samples). Charts were generated using gene length-normalised, zero-corrected abundance. "-Other" represents abundance data for all other AMR genes or drug classes in the abundance data set.

including urinary, enteric and respiratory infections[68]. It is thus not surprising that the data on antimicrobial use for the current study showed predominantly amoxicillin (beta-lactam) and co-trimoxazole (sulfonamide) use in the children. In addition, ceftriaxone and benzylpenicillin (both beta-lactams) and co-trimoxazole are among the most commonly used antibiotics in Zimbabwe[69].

Increased antimicrobial use impacts the gut microbiota[70,71] and is selective for AMR in populations[72,73]. The limited association of the obtainable antimicrobial use data with both the microbiome and resistome in the current study might be surprising. However, a study by Dethlefsen et al.[70] showed that a majority of the bacterial community that was depleted post-ciprofloxacin administration was restored after 4 weeks. Our antimicrobial use data were limited to antibiotic use within the immediate 6 months prior to sampling and was less heterogeneous, thus any marked differences in the microbiota may have been missed. Our findings are consistent with those from recent studies on global sewage samples, which have shown a much stronger association between socio-economic factors related to health, sanitation and education with the resistome, compared to antimicrobial use[55,74]. This seems more likely to be the case in low- and middle-income countries, where a high contagion––the spread of resistant strains and genes––between individuals may take place, and any antimicrobial use in one individual may have general effects on the population as a whole[75]. Thus, antimicrobial use explains some, but not all variation in AMR genes in this population[72,73], and improving such socio-economic factors may improve AMR.

Our study had a few limitations. The cross-sectional study design allowed characterisation of the gut microbiome and its relationship with *S. haematobium* infection at a single time point. A longitudinal study will inform on the dynamic relationship between the two, as well as the time course or developmental-related trends in the observed profiles. A longitudinal study would also give an indication of the dynamic features of the AMR genes and how they are associated with individual-specific data. Furthermore, relating the presence of AMR genes to measurable phenotypic resistance of bacteria would give a stronger indication of the clinical implications of the AMR genes present.

In conclusion, we characterised, through shotgun metagenomic sequencing, the microbiome (to include the fungi repertoire) and resistome in a preschool population (≤5 years old) in Zimbabwe. We identified differences in the gut microbiome between schistosome infected and uninfected children, showing largely an increase in abundance of specific bacteria, and for the first time (to our knowledge), fungi genera in infected children. This association was independent of age, sex and village. Mechanistic studies are required to further explain this relationship. To the best of our knowledge, we also characterised for the first time in this African preschool population, a diversity of AMR genes to bacteria, belonging to various functional drug classes. Our microbiome and resistome data add to publicly available data from different human populations.

Methods

Ethical approval and consent. The current study is part of a larger paediatric schistosomiasis study, for which ethical and institutional approval was obtained from the Medical Research Council of Zimbabwe (MRCZ/A/1964) and the

University of Edinburgh (fmutapi-0002), respectively. Permission to conduct the study was obtained from the Mashonaland Central Provincial Medical Director. The study aims and procedures were explained to all participants and their parents/guardians in their local language, Shona. Written informed consent was obtained from the participants' parents/guardians and recruitment was voluntary with participants free to withdraw from the study at any stage. For the current study, only samples from participants who consented to be part of this sub-study were used.

Study design, population and site. This cross-sectional study forms part of the baseline survey of a larger study on paediatric schistosomiasis conducted in the Shamva district, Northeast Zimbabwe, to compare the health benefits of early treatment of schistosome infections in preschool-aged children, 6 months to 5 years old[27]. In brief, children within this age group who were lifelong residents, and with no history of tuberculosis, malaria/fever, or recent major illness/surgery were invited to participate in the baseline survey and onward recruitment into the main cohort, if they met further criteria of being negative for *S. haematobium* by egg count and had no history of antihelminthic treatment. The study followed two groups of schistosome-negative children for new infections and then compared re-infection rates across two different regimens, following treatment of first schistosome infection.

For the current study, a subset of 116 stool samples (from 1–5 year olds) were selected from the baseline survey for microbiome analysis. To be recruited as part of this subset, participants had to consent for their stool samples to be used as part of the current study. A questionnaire was administered at the time of recruitment to gather metadata on socio-demography, growth and nutrition, and clinical history. Clinical records were checked to obtain history of antibiotic use, within the 6 months preceding acquisition of stool samples. Parents/carers were also interviewed to ascertain the health history of the children, and those who had any such history were excluded. Children were tested via parasitological diagnosis for *S. mansoni* and soil-transmitted helminths (STH) as part of the baseline survey and none who were positive were included in the subset of 116 children in the current study. The subset of children was thus selected based on (a) consent for microbiome analysis, (b) availability of socio-demographic data, (c) availability of parasitology samples (urine and stool samples), (d) availability of test results and clinical history and (e) no current episode of diarrhoea (assessed by questionnaire and visual stool examination).

Samples included in the current study were from two main sites, Mupfure and Chihuri. The sites are located in the Mashonaland Central province, with 123,650 people living in a 99% rural area of 2695 km^2, according to the 2012 national census[76]. The inhabitants are primarily subsistence farmers. The area has a high prevalence of *S. haematobium* (>50%) with low prevalence of *S. mansoni* and STH (<15%)[77], making it ideal for studies on the impacts of urogenital schistosomiasis.

Sample size. The samples used in the current study are from the baseline survey of a larger epidemiological study comparing re-infection rates across two different treatment regimens. As this is a relatively new field in human helminthology, there are limited published studies, with none focusing on the age group in the current study, i.e. 1–5 year olds. Thus there were no published baseline data to inform sample size calculations when we conducted our study. The sample size for the current study was informed by our previous study[35] and those of others[34,39] in older children with sample sizes ranging from 34–139, from which statistically significant differences were detected in the microbiome of schistosome infected versus uninfected children.

Sample collection, processing and DNA extraction. Urine and stool specimens were collected for parasitological diagnosis of *S. haematobium* and intestinal helminths, respectively. In summary, about 50 ml of urine sample was collected on three successive days, and a single stool sample was collected on a single day from each participant. Urine bags (Hollister 7511 U-Bag Urine Specimen Collector, Hollister Inc., Chicago, IL, USA) and disposable diapers (for stool samples) were used for sample collection in very young children. Urine samples were examined microscopically for *S. haematobium* infection following the standard urine filtration method[78], and stool samples examined microscopically using the Kato–Katz method[79], to exclude *S. mansoni* and STH (for at least one parasite egg detected). Infection intensity for *S. haematobium* was defined as the arithmetic mean egg count/10 ml of at least two urine samples collected on three consecutive days. All children who were positive for schistosome infection were treated with a single dose of praziquantel at the standard 40 mg/kg body weight, crushed and administered with squash and sliced bread[28] by local nurses.

Aliquots of stool samples in 2 ml cryotubes were stored temporarily at 2–8 °C for a maximum of 24 h prior to processing. For each stool sample, DNA was extracted using the QIAamp DNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions. To ensure sample aliquots contained purified DNA, each sample was quantified in-house at the University of Edinburgh using the Qubit fluorometer (ThermoFisher Scientific) prior to shipment for DNA sequencing.

Library preparation and sequencing. DNA samples were shipped on dry ice for library preparation and sequencing at the Beijing Genomics Institute (BGI, Shenzhen, China). At BGI, DNA from the stool samples was quantified using the Qubit fluorometer (ThermoFisher Scientific) and the NanoDrop™ spectrophotometer (ThermoFisher Scientific). As a quality control measure, the integrity and purity of DNA was assessed by a 1% agarose gel electrophoresis, run at 150 V for 40 min; DNA was sheared by ultrasonication into fragments (Covaris). Fragments were mixed with End Repair mix (BGI) and purified using the QIAquick PCR Purification Kit (Qiagen). Adapter-ligated DNA fragments were separated by electrophoresis through a 2% agarose gel to recover the target fragments, and purified using the QIAquick Gel Extraction kit (Qiagen). Library preparation to enrich the adapter-ligated DNA was done via PCR amplification, size-separated by electrophoresis, and purified using the QIAquick Gel Extraction kit (Qiagen). The final library was quantified using the Agilent 2100 bioanalyzer. The qualifying 116 DNA libraries were amplified using the cBOT system (Illumina), and sequenced on the Illumina Hiseq 4000 platform (Illumina) using paired-end 150-bp sequencing.

Bioinformatics processing: quality control and trimming. Raw FASTQ format sequences from each sample were quality assessed using FASTQC v0.10.0 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The number of read pairs generated per sample ranged from 9,263,538 to 21,350,613 [Supplementary Data 1]. Subsequently, reads were trimmed, to include removing adaptors, using BBduk2 [BBMap—Bushnell B.—https://sourceforge.net/projects/bbmap/] with an output quality Phred threshold score of ≥20 and a minimum read length of 50 bp. *K*-mer length for finding contaminants was $k = 19$. We looked for shorter *k*-mers at read pairs down to $k = 11$, and reads were trimmed at the right end [Supplementary Data 1].

Bioinformatics processing: mapping of sequence reads. Using a novel reference based mapping and alignment tool, *k*-mer alignment (KMA)[80], the trimmed reads were used as input to align directly against reference sequence databases. The KMA method is designed to improve mapping against redundant databases, and has been shown to outperform existing mapping methods in terms of speed, precision and sensitivity[80]. In summary, KMA, employs heuristic mapping, which involves directly mapping *k*-mers between query sequences and selected template databases, including large redundant databases. It then speeds up mapping by using *k*-mer seeding, and utilises a special version of the Needleman–Wunsch algorithm[81] to accurately align regions of mismatching *k*-mers. To ensure the best match template for the query reads, multi-mapping reads are resolved using a novel sorting scheme, ConClave. The scheme enables assembly of reads which results in a final accurate consensus sequence for the reference sequence, and also rules out bias associated with base calling across different sequencing platforms[82].

Bioinformatics processing: microbiome sequence component. To access the microbiome sequence component present in our samples, read pairs and singletons were aligned to a custom reference genomic database (last updated 04.04.2019). Mapped reads were counted as one copy, in cases of read pairs or singletons. Unless otherwise specified below, databases were primarily downloaded via NCBI GenBank clade specific assembly_summary.txt files (ftp://ftp.ncbi.nlm.nih.gov/genomes/genbank). The custom database consisted of the following: bacteria (closed genomes; downloaded 05.02.2019), archaea (downloaded 13.02.2019), MetaHitAssembly (PRJEB674–PRJEB1046; downloaded 01.07.2014), HumanMicrobiome (genome assemblies; downloaded 02.07.2014), bacteria_draft (downloaded 05.02.2019), plasmid (downloaded 05.02.2019), virus (https://bitbucket.org/genomicepidemiology/kvit_db; downloaded 05.02.2019; https://genome.jgi.doe.gov/portal/pages/dynamicOrganismDownload.jsf?organism=IMG_VR; downloaded 28.01.2019), fungi (downloaded 13.02.2019), protozoa (downloaded 13.02.2019), and parasites (downloaded 04.04.2019). Sequences selected for the bacteria and bacteria_draft databases from the assembly_summary.txt file were annotated with the tags version_status = "latest" and genome_rep = "Full". Additional entries, assembly_level = "Complete genome" or "Chromosome" in the bacteria database and refseq_category = "representative genome" in the bacteria_draft database were also required. The plasmid database was constructed as a subset of the bacteria and bacteria_draft sequences; keyword in the FASTA entry header line, "plasmid". The total read count for each microbial community of interest in a sample was calculated as the sum of read counts from each of the databases of interest; bacteria (bacteria, bacteria_draft, MetaHitAssembly, and HumanMicrobiome), fungi, protozoa, and parasites. Sequence mapping statistics are shown in Supplementary Data 1.

The primary (most similar) alignment obtained for mapped sequences was used to assign a putative taxonomy, based on the taxonID obtained. TaxonID's and associated taxonomy classifications were obtained from downloaded reference microbial genomes from NCBI (ftp://ftp.ncbi.nih.gov/pub/taxonomy/taxdump.tar.gz) and assignment at all taxonomic levels was done. Sequences that had no similarities detected in the nucleotide (nt) database for which we could not assign a putative taxonomic classification were deemed to be unknown sequences, and hence termed "Unknown". The classification of "unknown" is exquisitely time-sensitive, but was appropriate and correct at the time of this analysis. Details of taxonID mapping are shown in Supplementary Data 1.

Bioinformatics processing: AMR gene component. To identify any putative AMR genes present in the samples, the read pairs were aligned to AMR genes (3081

genes) present in the ResFinder database (https://bitbucket.org/genomicepidemiology/resfinder_db; downloaded 13.02.2019) with parameters set for a query gene to cover at least 2/5 the length of the reference gene to be selected[83]. Alignments were filtered to retain those exhibiting a selected threshold of identity of 90% (i.e. >90% nucleotide identity between the query and reference gene over at least 90% of the length of reference gene). Sequence mapping statistics are shown in Supplementary Data 1.

Data handling and processing. To account for probable sample-wise sequencing depth differences, as well as a size-dependent probability of observing a reference, mapping counts from the custom genomic database and from the ResFinder database were normalised to the total genome sizes for the genomic database and to the individual gene lengths for the ResFinder database (gene and genome size details in Supplementary Data 1).

The total observed mapping counts are relative, and may account for confounding effects on downstream analyses[84]. This may be due to limitations of an arbitrary total imposed by different sequencing platforms, technical variations in sequencing libraries amounts, or even random variation[85]. To obtain information about the abundances of features in our data set relative to each other, datasets were treated as compositional[85]. Data were transformed using the log-ratio approach as introduced by Aitchison[86], to make the data symmetric, linear and in a log-ratio coordinate space. However compositional methods such as this do not account for the presence of zeros associated with abundance datasets. To address this, a small pseudocount of half the smallest non-zero abundance per feature was added to each respective feature for all the normalised abundance matrices, prior to transformations[87]. Microbiota abundance data tables with counts, x, and k number of populations (taxa members), were centred log ratio (clr) transformed, defined as[87]

$$\mathrm{clr}(x_1, \cdots, x_k) = \left(\log\left(\frac{x_1}{g_{(x)}}\right), \cdots, \left(\log\left(\frac{x_k}{g(x)}\right)\right)\right),$$

where, $g(x) = (\prod x_i)^{1/k}$ is the geometric mean of the particular composition.

AMR gene abundances were additive log-ratio (alr) transformed, taking the bacterial component of the microbiome (x_k) as the reference as[87]

$$\mathrm{alr}(x_1, \cdots, x_k) = \left(\log\left(\frac{x_1}{x_k}\right), \cdots, \left(\log\left(\frac{x_{k-1}}{x_k}\right)\right)\right).$$

Unless otherwise stated, clr and alr matrices were used for all downstream analyses. Raw mapping count data and their corresponding alr and clr values for the analysed samples can be found in Supplementary Data 2.

Visualisation. Data visualisation was performed within the R environment (www.bioconductor.org; www.r-project.org). Bar plots from normalised, zero-corrected abundance matrices were used to give an overview of the microbiota and AMR gene abundances across all samples. For cluster dendrograms, the Aitchison distance (Euclidean distance) was calculated using clr-transformed abundance data, and samples clustered based on distances (complete-linkage-clustering). To explore underlying variabilities in the microbiota and AMR genes across the data set, clr-transformed abundance data for each matrix, centred on the geometric mean, scaled by the total variance were ordinated using PCA[87], based on eigenvectors and eigenvalues[88]. The PCA involves using multivariate data reduction techniques through linear combinations of variables (principal components), each of which explains a percentage variation[89]. Box plots were used to highlight differences in microbiota abundance between two groups, and scatter plots to show the relationship between schistosome infection intensity and microbiota abundance.

Statistics and reproducibility. Statistical analyses were performed using various Bioconductor packages within the R environment (www.bioconductor.org; www.r-project.org). To test whether sample-related metadata predict within-group dispersion of the microbiota and the AMR genes, the Euclidean distances were calculated, using the R/Bioconductor package vegan[90]. The effect of such metadata on sample dissimilarities were determined using permutational multivariate analysis of variance (PERMANOVA; adonis2 function in the vegan package) using $P < 0.05$ as the significance threshold. An FDR (Benjamini–Hochberg FDR) correction was applied to counteract multiple testing[91].

To investigate further how specific taxa composition vary across the statistically significant metadata (from PERMOANOVA), while controlling for other variables of interest, analysis of composition of microbiomes (ANCOM) was used[92]. ANCOM was the preferred choice because it does not make any distributional assumptions of the data. The algorithm computes log-ratios of raw count data (clr), where the normalising reference value is the abundance of all remaining taxa, taken one at a time. ANCOM uses bootstrapped intervals to perform hypotheses tests while maintaining the Benjamini–Hochberg FDR set at 0.05 (ref. [91]). A taxa member was considered varying in composition across an independent variable of interest when it varied across the independent variable of interest with respect to 80% of the rest of the taxa in the data set (W-statistic cutoff: 0.80). By definition, the W value generated (the number of times the null hypothesis is rejected for a given taxonomic group) is the ratio of a specific taxonomic group and a number of other groups (i.e. the W value) that are different across two groups. The ANCOM test for the influence of *S. haematobium* infection was controlled for age, sex and village.

As ANCOM only provides a list of taxa that vary in composition, the magnitude and direction of associations of taxa that vary in composition across independent variables was further determined. Box plots stratified by specific independent variables, using the clr-transformed abundance data of taxa previously identified as statistically significant by ANCOM were used to highlight differences in groups. To determine how these taxa varied with schistosome infection intensity, clr-transformed abundance data were regressed on the log transformed infection intensity ($\log_{10}$ [egg count + 1]).

The sample size used in the current study was based on availably of stool samples from the subset of children who gave consent and met the required selection criteria. Three samples were excluded from the overall analysis using a predefined criteria. To appropriately explain variations in the data, samples with non-missing data from at least one variable metadata from growth and nutritional indices, schistosome infection status, previous schistosome treatment and antibiotic use data (see Supplementary Data 1) were used for all downstream analysis (i.e. $n = 113$). Duplicate samples collected from two participants were used as biological/technical replicates for shotgun metagenomic sequencing.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Raw sequence data files from all 116 samples and associated metadata used in the current study are deposited in the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information (NCBI) database under the BioProject accession number PRJNA521455. In Supplementary Data 1, we present sample metadata and all summary statistics generated from the analyses of sequence reads. The source data underlying statistical analyses and figures are shown in Supplementary Data 2. All other data are available on request to the corresponding author.

Code availability

The updated R codes used for analysis of composition of microbiomes (ANCOM) are available on https://github.com/zellerlab/crc_meta/blob/master/src/ANCOM_updated.R OR from the author's webpage at https://sites.google.com/site/siddharthamandal1985/research.

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Author contributions

D.N.M.O., T.M., F.M.A., M.E.J.W. and F.M. conceptualised and designed the study. D.N.M.O., T.M., T.C., S.A.A., J.M. and F.M. were involved in the fieldwork, sample collection and DNA extractions. D.N.M.O. and T.C., curated the field data. D.N.M.O., J.M. and F.M. organised the sequencing. T.N.P. and A.I. carried out the bioinformatics processing. D.N.M.O., P.M., T.N.P., C.B. and A.I. analysed, produced figures and interpreted the data. D.N.M.O. prepared the draft manuscript and all authors were involved in review, editing and approval of the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Six rounds of annual praziquantel treatment during a national helminth control program significantly reduced schistosome infection and morbidity levels in a cohort of schoolchildren in Zimbabwe

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Data Availability Statement: All the treatment coverage data are publicly available on the ESPEN data base on: http://espen.afro.who.int/ The infection level data belong to a third party, the Ministry of Health in Zimbabwe as per requirements of our ethical approval conditions. Upon publication, the Ministry of Health will deposit the data in the WHO public data base ESPEN. Other parties can also apply for the data from the Director of the Medical Research Council

Abstract

Background

The World Health Organization recommends that schistosomiasis be treated through Mass Drug Administration (MDA). In line with this recommendation, Zimbabwe commenced a national helminth control program in 2012 targeting schoolchildren throughout the country for 6 years. This study, part of a larger investigation of the impact of helminth treatment on the overall health of the children, determined the effect of annual praziquantel treatment on schistosome infection and morbidity in a cohort of children during Zimbabwe's 6-year national helminth control program.

Methodology/Principal findings

A school-based longitudinal study was carried out in 35 sentinel sites across Zimbabwe from September 2012 to November 2017. The sentinel sites were selected following a countrywide survey conducted in 280 primary schools. *Schistosoma haematobium* was diagnosed using the urine filtration technique. *Schistosoma mansoni* was diagnosed using both the Kato-Katz and formol-ether concentration techniques. *S. haematobium* morbidity was determined through detection of macro and microhaematuria. A cohort of children aged 6–15 years old was surveyed annually before MDA and 6 weeks post treatment. Maximum treatment coverage reached 90% over the 6 rounds of MDA. At baseline *S. haematobium*

of Zimbabwe http://www.mrcz.org.zw/, the body granting ethical approval for the study. They can do so via the MRCZ contact portal on: http://www.mrcz.org.zw/contact-us/.

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infection prevalence and intensity were 31.7% (95% CI = 31.1–32.2) and 28.75 eggs/10ml urine (SEM = 0.81) respectively, while *S. mansoni* prevalence and intensity were 4.6% (95% CI = 4.4–4.8) and 0.28 eggs/25mg (SEM = 0.02). Prior to the 6th round of MDA, *S. haematobium* infection prevalence had reduced to 1.56% (p<0.001) and infection intensity to 0.07 (SEM 0.02). Six weeks later after the 6th MDA, both were 0. Similarly the prevalence of *S. haematobium* morbidity as indicated by haematuria also fell significantly from 32.3% (95% CI = 29.9–34.6) to 0% (p< 0.0001) prior to the final MDA. For *S.mansoni*, both prevalence and intensity had decreased to 0 prior to the 6th MDA. After 6 rounds of annual MDA, prevalence and intensity of both schistosome species decreased significantly to 0% (p< 0.0001).

Conclusion

Zimbabwe's helminth control program significantly reduced schistosome infection intensity and prevalence and urogenital schistosomiasis morbidity prevalence in a cohort of school-aged children, moving the schistosome prevalence in the children from moderate to low by WHO classification. These findings will inform the design of the country's next stage interventions for helminth control and eventual elimination.

Author summary

Following a national helminth prevalence survey conducted in 2010/2011, Zimbabwe launched a national schistosomiasis and intestinal worm control program in 2012 targeting school children aged 6–15 years who carry the highest infection burden. The control strategy as advocated by the World Health Assembly in 2012 was adopted to suit the country context. We conducted a study in a cohort of children to assess the effect of praziquantel treatment on schistosome infection intensity and prevalence as well as on urogenital schistosomiasis morbidity prevalence after every annual MDA for 6 years. Maximum treatment coverage in a cohort of children reached 90% over the 6 rounds of annual treatment. Annual surveys in sentinel sites throughout the country showed that schistosome infection prevalence and intensity as well as the marker of urogenital schistosomiasis, haematuria decreased significantly after each round of treatment from the pre-treatment levels in the same year. Most notably, both urogenital schistosomiasis infection and morbidity prevalence reduced from 32% at baseline in 2012 to 1.56% before the 6th MDA in 2017, and infection intensity reduced from 28.75 eggs/10ml urine to a mean of 0.07eggs/10ml urine prior to the 6th MDA. Both infection prevalence and intensity fell to 0 after the 6th MDA. Intestinal schistosomiasis prevalence and intensity reduced from 4.6% and 0.28 eggs/25mg of stool to 0% and 0 eggs, respectively. Thus, the control program significantly reduced schistosome infection and morbidity in this cohort of schoolchildren.

Introduction

Zimbabwe is endemic to several of the neglected tropical diseases (NTDs) as listed by the World Health Organization (WHO) [1]. Of these NTDs, schistosomiasis is the most prevalent in the country. Both forms of schistosomiasis; urogenital and intestinal, occur in Zimbabwe with the former being more prevalent [2]. From the very first community survey in Zimbabwe,

then Rhodesia, in 1932 [3], Zimbabwe has had programs to control schistosomiasis [4]. These programs have targeted workers and schoolchildren using several approaches including treatment of infected people (first with tartar emetic and then praziquantel(PZQ)), improving water and sanitation, snail control and education/awareness campaigns [4]. However, these programs have mostly been focal in space and time [5–8]. In 2012 the WHO set out a roadmap for NTDs, including controlling schistosomiasis morbidity by 2020 and elimination of schistosomiasis as a public health problem and interrupting transmission in various African countries by 2025 [9]. As part of implementing this roadmap, Zimbabwe has been conducting a national helminth control program targeting school children aged 6 to 15 years, whom previous studies in the country have shown to carry the heaviest burden of schistosome infection in Zimbabwe [8, 10–13].

To inform the national control program, we performed a nationwide school-based survey in 2010/2011, mapping *S. mansoni*, *S. haematobium*, and the soil-transmitted helminth species: *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm [2]. This was done by collecting data at district level, and thereby representative of the corresponding provinces. Data from this study revealed high schistosomiasis prevalence in 55 districts and soil transmitted helminths occurring in 47 districts, with 33 districts being endemic to both types of NTDs. The nationwide combined prevalence of schistosomiasis was 22.7% while that for STH was 5.5% [2]. These levels of infection demanded urgent interventions. Following this, the Ministry of Health formulated a national NTD control policy, an initial 5-year action plan and implemented school-based mass drug administration (MDA) targeting primary and secondary school children across the country [4]. Crucially, Zimbabwe's control program treated all children annually irrespective of the regional prevalence of infection. This is in contrast to the WHO preventive chemotherapy guidelines that recommend the frequency of treatment based on community/regional infection level, i.e. high risk communities, those with a prevalence of 50% and over, should be treated annually, whereas those at lower risk should be treated every two years (over 10% but less than 50%). In cases where prevalence was less than 10%, then the recommendation is that children should be treated twice within their school career [14] [15].

While data on the coverage of the country's helminth control program has been documented (see http://espen.afro.who.int/countries/zimbabwe), there had been no indication of the effectiveness of the control program in terms of efficacy of praziquantel treatment on infection intensity and prevalence as well as on levels of urogenital schistosomiasis morbidity as measured by haematuria. This study is part of an investigation on the impact of schistosomiasis treatment with PZQ during the national control program on overall human health including morbidity markers, inflammatory markers, allergic responses and autoreactivity in school children. For this study, we determined egg reduction rates (ERR) and cure rates (CR) in a cohort of school children. This current study focuses on the effect of annual PZQ treatment on schistosome infection intensity and prevalence and urogenital schistosomiasis morbidity in this cohort of school children during Zimbabwe's national helminth control program and compares infection levels from the start of the MDA to our last survey six weeks after the 6th round of MDA.

Materials and methods

Ethical approval and consent

The study received institutional approval from the University of Zimbabwe and Ethical approval from the Medical Research Council of Zimbabwe MRCZ/A/1710. Permission to conduct the study in the provinces was obtained from the Ministry of Health & Child Care.

Recruitment into the study was voluntary and parents/guardians gave written parental consent and were free to withdraw the participants at any time with no further obligation.

Background and national MDA

Between September 2010 and August 2011, a national schistosomiasis and soil transmitted helminths survey was conducted in Zimbabwe by the Ministry of Health and Child Welfare through the Epidemiology and Disease Control Department and National Institute of Health Research with support from the Ministry of Education, the WHO and UNICEF [2].

In keeping with the observation that school children constitute the high-risk age group for schistosomiasis and STH in the community [14], the survey was conducted in school children aged 10–15 years old. The survey was a countrywide cross-sectional survey carried out by sampling 50 children in each of 280 primary schools in all the 68 districts of the country's total eight provinces. The national survey indicated the different districts in the country which fell in the WHO classification of heavy, moderate and low schistosome endemicity based on the infection levels in the school children [15].

Following this national survey, annual MDAs were carried out in September 2012, October 2013, January 2015, November 2015, November 2016 and November 2017. The third MDA was delayed due to logistical reasons, resulting in the reduced gap between the third and fourth MDA being less than 12 months. The children in the cohort study described here were treated during these MDAs.

Study design

The study presented here was designed to follow a cohort of primary school children who were targeted by this MDA from September 2012 to November 2017. Sites throughout the country were purposely chosen before the commencement of the MDA program to represent the 3 levels of schistosomiasis infection prevalence as described by WHO i.e. low, moderate and high [14] at both district and province level as shown in **Fig 1**. The sentinel sites were also chosen to represent the baseline prevalence classification of the district (low, medium or high) (see **S1 Fig**). In total 35 schools located in 29 districts and 8 provinces were included in the study. The sample sizes are given in **Table 1** and the map of the 8 provinces is given in **Fig 2A.**

Of the 8 provinces, 6 had at least one sentinel site included in every MDA, while 2 provinces were not included in all the surveys. Matabeleland South and Mashonaland West were not surveyed in all MDAs. The national survey showed that Matabeleland South had the lowest schistosomiasis prevalence in the country [2] and it was deemed not cost effective to survey this province after every MDA. Therefore, this province was surveyed at the start of this project in 2012 and at the end in 2017, i.e. pre- and post-MDA 1 and post-MDA 6. Similarly other sites including all those in Mashonaland West were sampled at the start-point (MDA1), mid-point (MDA4) and end-point (MDA6) of the control program. These are detailed in **S1 Fig** and in **Table 1.** In the remaining sentinel sites children were surveyed annually for the duration of the study.

For each MDA, the school children were sampled at two points, prior to treatment (pre-MDA) and 6 weeks after treatment was given (post-MDA). Twelve schools (sentinel sites; Gonye, Siabuwa, Muzarabani, Kafura, Chiguri, Mukwakwe, Negato, Mareya, Ruzongwe, Mbembi, Bandanyenje, Kanyaga) (see **Fig 2B**) were followed up annually. As indicated in the study design in **Fig 1**, these 12 schools were purposely selected to represent the full range of the different levels of baseline schistosomiasis infection prevalence i.e. low, moderate and high.

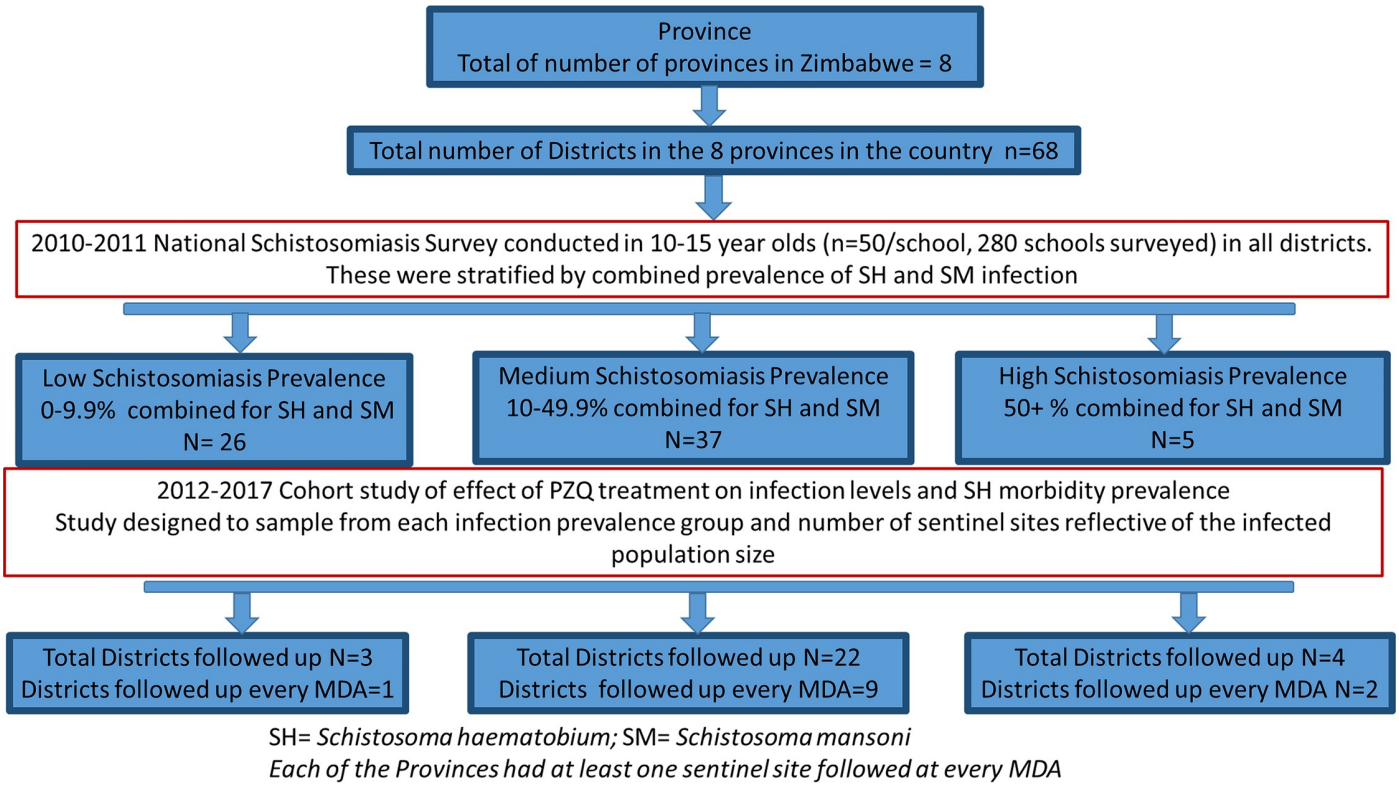

Fig 1. Study design. The schistosome endemicity levels (low, medium, high) were stratified based on combined *S. mansoni* and *S. haematobium* infection prevalence.

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Study population

The study recruited children aged 6–15 years old and these children would typically be in grades 3–7 in primary school. The children were randomly selected prior to the start of the study in 2012 and the same children were followed up for the duration of the study i.e. until 2017. The selected children were sampled at each sampling point regardless of whether they had received treatment in the last MDA or not. The treatment records of the children were extracted during the post-MDA visit for each MDA, from the MDA records kept by the school. These records informed the treatment coverage rates. Children leave primary school in Zimbabwe for senior school after grade 7, therefore, children who moved out of grade 7 were followed up if enrolled at a local secondary school to ensure the post treatment follow-up. The majority of children lost to follow-up were those who had completed primary school and moved on to secondary school as well as those who transferred to other distant schools.

Inclusion criteria

To be included in the study, children had to i) provide all 2 stool samples and 3 urine samples for schistosome parasitology and morbidity determination at pre-treatment and 6 weeks post-treatment survey points, ii) have been offered treatment with PZQ during that year's MDA.

Study sample sizes

Our national survey sampled 50 children from each school based on the sample size calculation previously published which gave a total sample size of 15, 818 [2] for the national survey.

Table 1. Summary study sample sizes by MDA.

Province	District	School	Total number of surveys	Number of children surveyed per MDA					
				1	2	3	4	5	6
Manicaland	Bikita	Chitenderano	2	195	-	-	-	-	195
Manicaland	Buhera	Masocha	6	242	-	-	242	-	242
Manicaland	Chipinge	Chitepo	6	250	-	-	250	-	250
Manicaland	Makoni	Bandanyenje	12	242	242	211	242	242	193
Manicaland	Nyanga	Chipataronga	6	398	-	-	398	-	398
Mashonaland Central	Guruve	Nyanhunzi	6	179	-	-	179	-	179
Mashonaland Central	Mazowe	Mbebi	6	186	-	-	186	-	186
Mashonaland Central	Mt Darwin	Bemberi	12	201	206	206	222	196	145
Mashonaland Central	Muzarabani	Muzarabani	12	298	297	298	298	298	213
Mashonaland Central	Rushinga	Mazowe Valley	12	250	250	250	250	250	208
Mashonaland Central	Shamva	Chihuri	6	218	-	-	218	-	218
Mashonaland Central	Shamva	Gono	3	200	-	-	-	-	200
Mashonaland East	Chikomba	Nhidza	6	250	-	-	250	-	250
Mashonaland East	Murewa	Chiguri	12	198	198	198	198	198	175
Mashonaland East	Murewa	Chingono	6	224	-	-	224	-	224
Mashonaland East	Mutoko	Chimukopa	12	250	198	224	224	224	201
Mashonaland East	Mutoko	Kushinga	6	198	-	-	198	-	198
Mashonaland East	UMP	Kafura	12	250	250	250	250	250	226
Mashonaland West	Chegutu	Gadzema	6	267	-	-	267	-	267
Mashonaland West	Hurungwe	Dandawa	6	206	-	-	206	-	206
Mashonaland West	Hurungwe	Gache-gache	6	233	-	-	233	-	233
Mashonaland West	Makonde	Kanyanga	6	250	-	-	249	-	249
Mashonaland West	Zvimba	Chomutamba	3	247	-	-	-	-	247
Masvingo	Chiredzi	Mareya	12	220	218	218	218	218	200
Masvingo	Gutu	Mutendeure	3	192	-	-	-	-	192
Masvingo	Mwenezi	Rudzongwe	12	267	267	267	267	267	210
Matabeleland North	Binga	Siabuwa	3	233	-	-	-	-	233
Matabeleland North	Nkayi	Gonye	12	242	242	211	242	242	193
Matabeleland North	Insiza	Insiza	3	248	-	-	-	-	240
Midlands	Chirumanzu	Gambiza	3	123	-	-	-	-	123
Midlands	Gokwe North	Chireya	3	192	-	-	-	-	192
Midlands	Mberengwa	Mukwakwe	12	177	179	160	179	179	156
Midlands	Mberengwa	Nhemga	3	250	-	-	-	-	248
Midlands	Mberengwa	Zverenje	3	253	-	-	-	-	253
Midlands	Shurugwi	New Gato	12	186	186	186	186	186	186

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Briefly, in that national survey calculation using 37% as the assumed mean prevalence of schistosomiasis and the error margin of 0.75%, the number of schools selected per district was determined by dividing the district sample size calculated proportionally from the national sample size by the number of children that would be screened per school (n = 50). In this survey, we based our study design on that original study design for consistency and our sample sizes were informed by the original countrywide survey. The present study was to follow the children for 6 years, we were cognizant of the possibility of loss due to migration. Thus, using the national sample size calculation we aimed to recruit a sample size of 250 children per school, with a minimum school sample size of 150 to give a total of 8750 children which would be 55% of the number of children sampled in the national survey. At baseline, a total of 8015

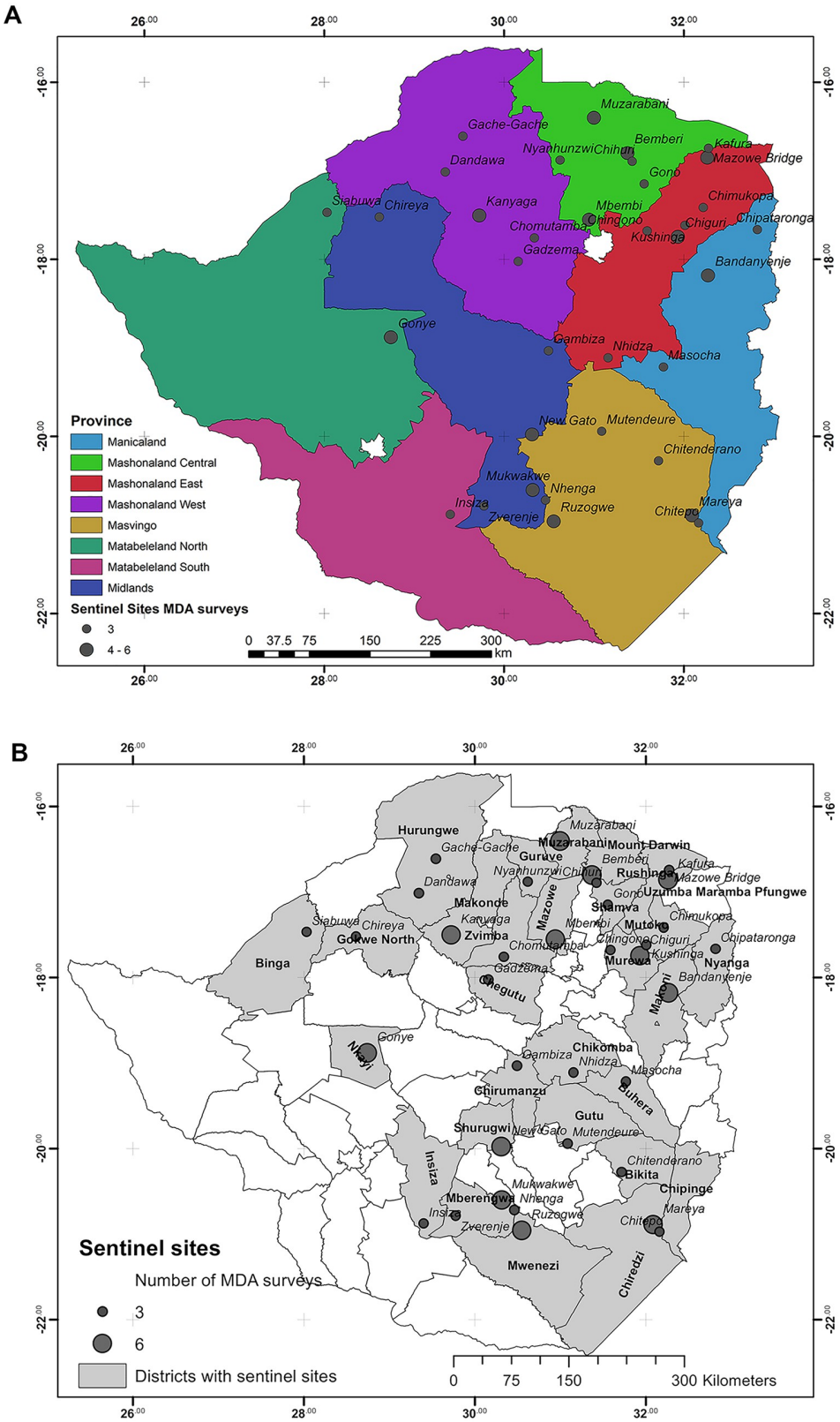
A
Province
Manicaland
Mashonaland Central
Mashonaland East
Mashonaland West
Masvingo
Matabeleland North
Matabeleland South
Midlands
Sentinel Sites MDA surveys
3
4 - 6
0 37.5 75 150 225 300 km
Muzarabani
Gache-Gache
Bemberi
Kafuta
Mazowe Bridge
NyanhunzwiChihuri
Dandawa
Gono
Siabuwa
Chireya
Kanyaga
Mbembi
Chimukoma
Chomutamba
Chingono
Chiguri
Chipataronga
Kushinga
Gadzema
Bandanyenje
Gonye
Gambiza
Nhidza
Masocha
New Gato
Mutendeure
Chitenderano
Mukwakwe
Nhenga
Insiza
Ruzogwe
Mareya
Zverenje
Chitepo
B
Hurungwe
Muzarabani
Mount Darwin
Guruve
Rushinga
Uzumba Maramba Pfungwe
Makonde
Shamva
Mazowe
Mutoko
Zvimba
Goromwe North
Murewa
Nyanga
Binga
Chiegutu
Makoni
Nkayi
Chikomba
Buhera
Chirumanzu
Gutu
Shurugwi
Insiza
Bikita
Chipinge
Mberengwi
Mwenezi
Chiredzi
Sentinel sites
Number of MDA surveys
3
6
Districts with sentinel sites
0 75 150 300 Kilometers

Fig 2. Maps showing sentinel sites at different administration levels. The maps were generated using GIS raw data for the schools using ArcMap 10.1. Sentinel sites are italicized and the number of MDA surveys indicated**. A.** Map showing sentinel sites at province level. **B.** Maps showing sentinel sites at different administration levels. The maps were generated using GIS raw data for the schools using ArcMap 10.1. Map showing sentinel sites at district level.

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children satisfied the eligibility criteria. Of these children, 7529 (94%) were followed up at the last survey after MDA 6. Of the children from the 12 sentinel sites followed every year, 2781 children were recruited, with 2306 (83%) followed up. Annual sample sizes are summarised in Table 1. Overall there was a loss to follow-up of 6% from MDA1 to MDA6.

Parasitology

Stool and urine samples were collected from each child between 1000 and 1400 hours, the period when peak egg excretion is expected [16], *S. mansoni* infection status and intensity were determined by microscopic examination of slides prepared using the Kato-Katz technique [17] and the formol-ether concentration technique [18] for collecting parasite eggs from stool samples. Two stool samples were collected from each individual over two consecutive days. A single slide was prepared following the Kato-Katz procedure using the 41.7mg template from each stool sample. The egg count from the 41.7mg template was multiplied by 24 as per WHO protocol guidelines (https://www.who.int/medical_devices/diagnostics/selection_in-vitro/selection_in-vitro-meetings/00054_01_kato-katzBench_aids.pdf). The participant's egg count was the arithmetic mean calculated from the two slides and recoded as the mean eggs per gram of stool. Stool left over from one stool sample was used for the formol-ether concentration technique [18]. There were no indicators for *S. mansoni* morbidity assessed due to lack of a point of care rapid diagnostic.

For *S. haematobium* infection status and intensity, a urine sample was collected from each participant on three consecutive days. 10mls of this urine was processed following the urine filtration method [19]. Thus, from each participant the three slides were prepared one from each day. The individual's egg count was the arithmetic mean calculated from the three slides. *S. haematobium* morbidity was determined by detection of blood in urine using dipsticks, presence of blood in urine in *S. haematobium* endemic areas is also used as indicative of *S. haematobium* infection [20]. Individuals were only considered positive if schistosome eggs were detected in their urine/stool samples.

Antihelminthic treatment

As previously indicated, the treatment was administered by the National Helminth Control treatment team as part of the annual national helminth MDA program. Children were measured using the PZQ dose poles to determine treatment dose and regardless of infection status, were co-administered a standard dose of PZQ i.e. 40mg/kg and albendazole at 400mg per child. During treatment, children were checked to confirm they had swallowed the tablet, and were given bread and juice. Treatment was administered by nurses and the school health coordinators, as is the Ministry of Health practice in Zimbabwe for the national helminth control program. The number and type of tablets administered was recorded for each child in MDA registers, for accurate recording of the MDA reach as well as to distinguish non-compliance from treatment failure in subsequent follow-ups.

Treatment coverage data

Summary treatment coverage data were available at program level for the country's MDA program annually for the duration of the study. In 2017, these data were present at district level

and the findings of the study were related to these data. All children followed up in the study were confirmed to have received PZQ treatment at each MDA.

Statistical analysis

Data recorded on field sheets was double entered and proof read in an Excel spreadsheet and comprised data from MDA 1 to 6 with both pre-MDA and post-MDA data recorded for each individual. Infection prevalence and intensity for each time point was calculated for each district.

To determine the effect of treatment on infection level, the ERR, a measure of the change in parasite egg burden upon treatment, and the CR, a measure of those cured of infection upon treatment, were calculated using data from treated children (as confirmed by the school MDA registers) who were positive for schistosome infection using Eqs 1–4 below.

$$\text{Infection intensity} = \frac{\text{Arithmetic Mean egg count for all individuals}}{\text{Total number of individuals sampled}} \quad \text{Eq 1}$$

$$\text{Prevalence} = \left(\frac{\text{Number of individuals positive for infection}}{\text{Total number of individuals sampled}}\right) \text{x } 100 \quad \text{Eq 2}$$

$$\text{ERR (\%)} = \left(1 - \left(\frac{\text{Arithemtic mean infection intensity following Rx}}{\text{Arithmetic mean infection intesity in infected people at baseline}}\right)\right) x100 \quad \text{Eq 3}$$

$$CR\ (\%) = \left(\frac{\text{Number of individuals positive at baseline and negative following } R_x}{\text{No of individuals positive at baseline}}\right) \text{x}100 \quad \text{Eq 4}$$

where: ERR = egg reduction rate, CR = cure rate, R_X = treatment.

Prevalence data and infection intensities were analysed using Minitab Statistical Software 18 and GraphPad Prism 8. Due to the non-parametric nature of the data, differences in prevalence between annual MDAs were tested for significance using a Fisher's exact test, whereas difference between pre- and post-MDA time points were analysed using a paired McNemar's test [21]. Infection intensities between annual MDAs were tested using an unpaired Student's t-test following Log_{10} (X+1) transformation to normalise data, and between pre- and post-MDA a paired two-way Student's t-test was calculated. For all analyses, a value of $p<0.05$ was considered significant. Infection prevalence maps were generated using this primary raw data and plotted using the software package ArcMap 10.1.

Results

Effect of treatment in the school children cohort at national level

Prior to the MDA, *Schistosoma haematobium* prevalence in the sentinel sites was 31.7% and, consistent with Zimbabwe's 2010 national schistosomiasis survey. *S. haematobium* was the most prevalent schistosome species in the country [2]. Following 6 annual rounds of MDA, the prevalence of *S. haematobium* decreased significantly to 0% ($p<0.0001$) (Fig 3A). When comparing infection prevalence pre-treatment, the pre-MDA 1 prevalence of 31.7% decreased to 1.56% ($p<0.001$) at pre-MDA 6 i.e. 12 months after the last MDA allowing for a full reinfection cycle (Fig 3A).

Schistosoma mansoni was tested for using both the Kato-Katz and formol-ether technique, and the prevalence resulting from each at baseline was found to be 4.6% and 1.4% respectively. The results of the more widely used Kato-Katz diagnostic are shown in Fig 3B. After the last

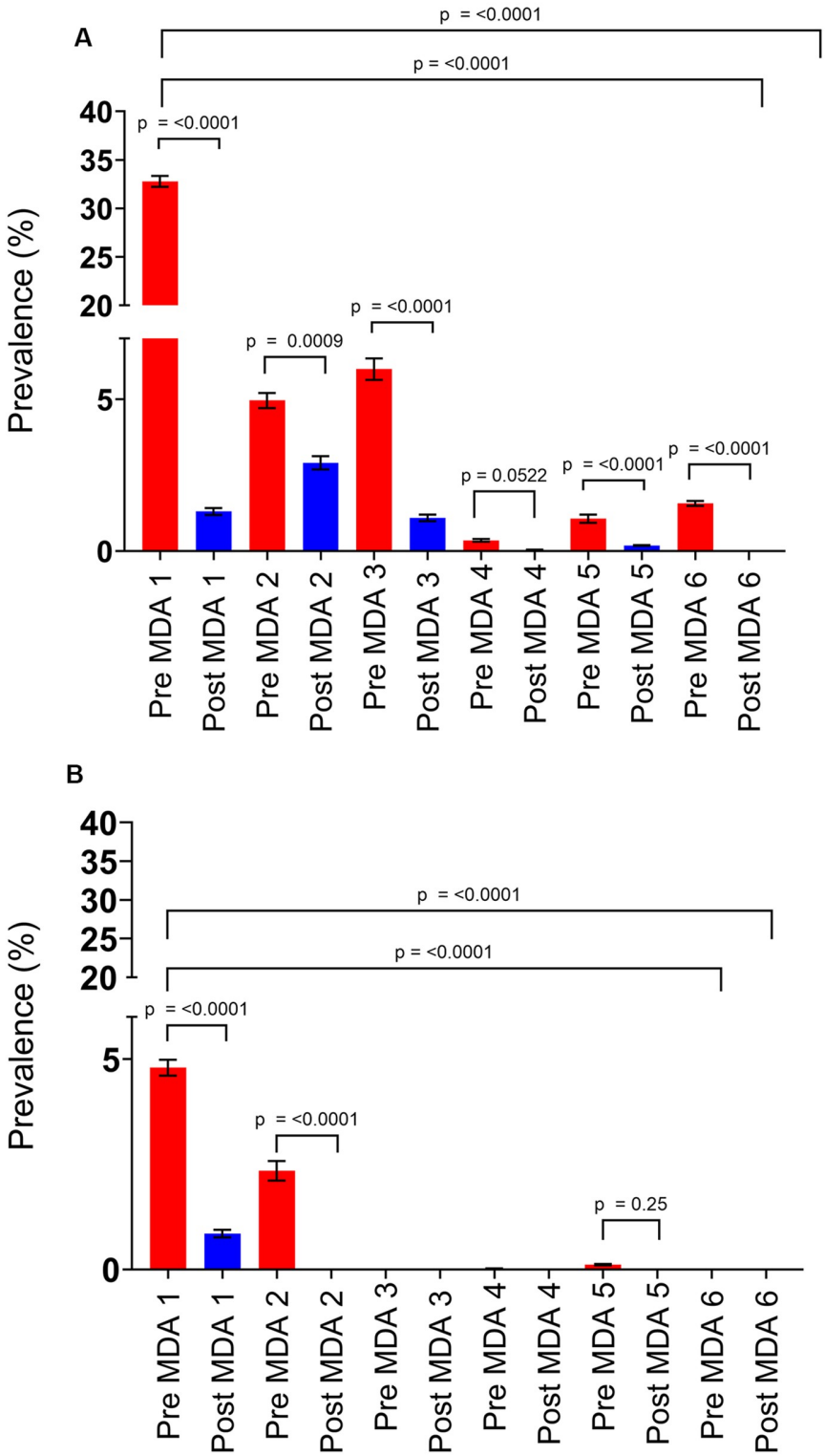
A
p = <0.0001
p = <0.0001
p = <0.0001
p = 0.0009
p = <0.0001
p = 0.0522
p = <0.0001
p = <0.0001
Prevalence (%)
40
35
30
25
20
5
0
Pre MDA 1
Post MDA 1
Pre MDA 2
Post MDA 2
Pre MDA 3
Post MDA 3
Pre MDA 4
Post MDA 4
Pre MDA 5
Post MDA 5
Pre MDA 6
Post MDA 6
B
p = <0.0001
p = <0.0001
p = <0.0001
p = <0.0001
p = 0.25
Prevalence (%)
40
35
30
25
20
5
0
Pre MDA 1
Post MDA 1
Pre MDA 2
Post MDA 2
Pre MDA 3
Post MDA 3
Pre MDA 4
Post MDA 4
Pre MDA 5
Post MDA 5
Pre MDA 6
Post MDA 6

Fig 3. Decline in overall infection prevalence in the cohort of children during the MDAs. Red bars = pre-treatment infection prevalence for each MDA. Blue bars = post-treatment infection prevalence for each MDA. **A**. *S. haematobium*. **B**. *S. mansoni* as diagnosed through the Kato-Katz procedure during the MDAs. Red bars = pre treatment infection prevalence for each MDA.

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MDA in 2017, *S. mansoni* prevalence was 0% when tested by both diagnostic techniques ($p<0.0001$). When comparing infection prevalence pre-treatment, the pre-MDA 1 prevalence of 4.6% by Kato-Katz decreased to 0% ($p<0.0001$) at pre-MDA 6 and from 1.4% at pre-MDA 1 by formol-ether also to 0% at pre-MDA 6 (see Fig 3B for the Kato-Katz data).

For both causative species of human schistosomiasis in Zimbabwe, *S. haematobium* and *S. mansoni*, arithmetic mean infection intensity (analysed using the Log_{10}(mean egg count+1) transformation) in the cohort of school children decreased significantly from the initiation of the MDA programme to the end ($p = <0.0001$) (see Fig 4). *S. haematobium* decreased from a mean of 0.49 (SEM = 0.01) to 0.07 (SEM 0.02) at pre-MDA 6 (see Fig 4A) and 0 (SEM = 0) at post-MDA 6. *S. mansoni* infection intensity determined by Kato-Katz decreased from 0.03 (SEM = 0.002) at pre-MDA 1 to 0 (SEM = 0) at both pre- and post-MDA 6, and from 0.006 (SEM = 0.0007) at pre-MDA 1 to 0 (SEM = 0) again at both pre- and post-MDA 6 using the formol-ether diagnostic technique (4b).

Schistosome morbidity

Macro or visible haematuria measured as a marker of morbidity of *S. haematobium* infection fell significantly from 32.3% in pre-MDA 1 to 0% at post-MDA 6 ($p <0.001$) and the gradual decline is shown in **Fig 5**. The prevalence of haematuria had fallen to 0% prior to the 6th MDA.

Effect of treatment in the school cohort at province level

The impact of treatment was investigated in all 8 provinces. The study shows that the first MDA had a significant effect on infection levels. Investigations in the sentinel sites in the 6 provinces surveyed at every MDA for the 6 MDAs shows a gradual decline in infection level. Fig 6A shows the decline in *S. haematobium* infection prevalence while Fig 6B shows the decline in *S. mansoni* prevalence as assessed by Kato-Katz.

Matabeleland North started with a prevalence of 5.3% for *S. haematobium* and 1.5% for *S. mansoni* in 2012 and decreased in both species to 0% by 2017. Masvingo had the highest starting prevalence among the provinces, calculated at 51.9% for *S. haematobium* and 16.1% for *S. mansoni* in 2012. These both decreased to 0% following the last MDA in 2017. The prevalence of *S. haematobium* in the Midlands was 32.3% in 2012 decreasing to 0% in 2017 and S. *mansoni* prevalence in the Midlands started at 0% in 2012, and remained at 0% in 2017. Mashonaland West province had a prevalence of 18.1% for *S. haematobium* and 2.1% in *S. mansoni* in 2012, both of which decreased to 0% in 2017. Mashonaland Central had a prevalence of 36.4% for *S. haematobium* and 3.7% for *S. mansoni* in 2012, and again, both decreased to 0% in 2017. Mashonaland East had an initial prevalence of 30.4% for *S. haematobium* and 3.9% for *S. mansoni*. These decreased to 0% after the 6th MDA. Manicaland province had a prevalence of 35.6% for *S. haematobium* and 8.8% for *S. mansoni* before the first MDA, and both decreased to 0% after the last MDA. Overall, the final data collected following the 2017 annual MDA revealed that every province had decreased to 0% for both *S. haematobium* and *S. mansoni*.

The overall change in prevalence between pre-MDA 1 and post-MDA 6 was significant in both *S. haematobium* ($p = <0.0001$) and *S. mansoni* ($p = <0.0001$) (Fig 2). When investigating this at a province level, the difference in prevalence of *S. haematobium* was significant for

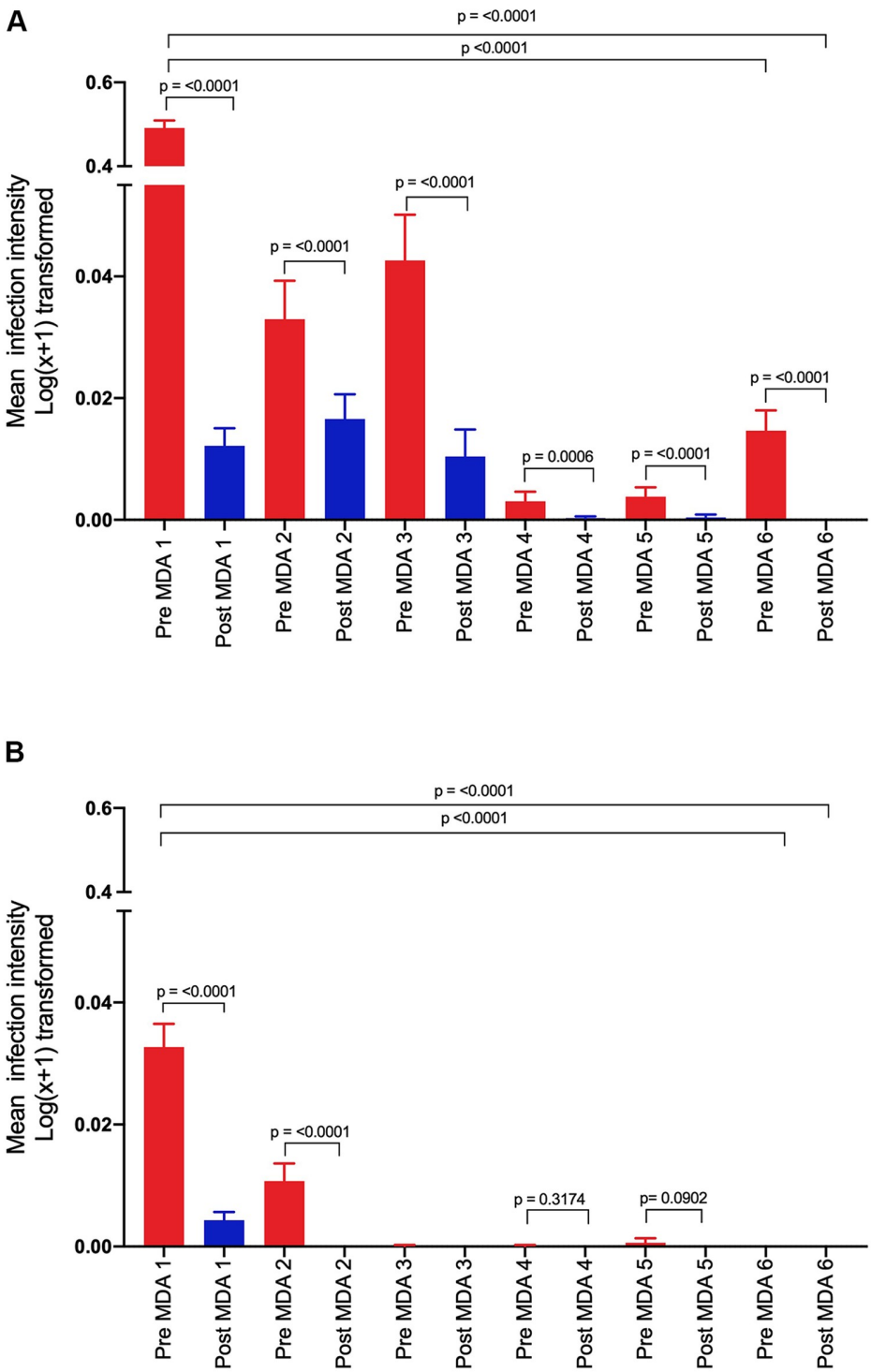
A
p = <0.0001
p <0.0001
p = <0.0001
p = <0.0001
p = <0.0001
p = 0.0006
p = <0.0001
p = <0.0001
Mean infection intensity Log(x+1) transformed
0.6
0.4
0.04
0.02
0.00
Pre MDA 1
Post MDA 1
Pre MDA 2
Post MDA 2
Pre MDA 3
Post MDA 3
Pre MDA 4
Post MDA 4
Pre MDA 5
Post MDA 5
Pre MDA 6
Post MDA 6
B
p = <0.0001
p <0.0001
p = <0.0001
p = <0.0001
p = 0.3174
p= 0.0902
Mean infection intensity Log(x+1) transformed
0.6
0.4
0.04
0.02
0.00
Pre MDA 1
Post MDA 1
Pre MDA 2
Post MDA 2
Pre MDA 3
Post MDA 3
Pre MDA 4
Post MDA 4
Pre MDA 5
Post MDA 5
Pre MDA 6
Post MDA 6

Fig 4. Decline in overall *S. haematobium* infection intensity during the MDAs in the cohort of children. Red bars = pre-treatment infection intensity for each MDA. Blue bars = post treatment infection intensity for each MDA. **A.** *S. haematobium*, **B.** *S. mansoni* infection intensity as measured by Kato-Katz.

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Mashonaland West, East, Central, Midlands, Masvingo, Manicaland (all p = <0.0001) and Matabeleland North (p = 0.0002). The change in prevalence of *S. mansoni* on the province level was also significant in Mashonaland East, West, Central, Manicaland and Masvingo (all p = <0.0001), however Matabeleland North was not (p = 0.2000) and Midlands had no detectable *S. mansoni* at either time points. The impact of the MDAs on both schistosome species is shown in **S2 Fig**.

Effect of treatment in the school cohort at district level

Of the 29 districts investigated in MDA 1, 24 (82.8%) districts were positive for *S. haematobium*, 33.3% for both and 10 (34.5%) districts were positive for *S. mansoni*. District prevalences ranged from 0% to 88.1% pre-MDA 1 for *S. haematobium* and 0% to 25.5% for *S. mansoni* as shown by the data of pre-MDA 1 prevalences in Fig 6A for *S. haematobium* and Fig 6B for *S. mansoni*. Infection prevalence in all districts was 0% post-MDA 6 for both species.

The summary of the overall impact on national prevalence in the cohort of children, presenting the initial data collected prior to the commencement of the first MDA in 2012, to the final measurements taken following the 6th MDA in 2017 is shown in Fig 7.

Egg reduction and cure rates

The egg reduction rates (ERR) and cure rates (CR) were calculated for each MDA. Overall ERR and CR are given in Tables 2 and 3 respectively. Across all provinces, for both *S. mansoni* and *S. haematobium*, ERR were all above 90%. Across the MDAs, average CR ranged from 83.4% to 100% for *S. haematobium* and 92.8% to 100% for *S. mansoni* diagnosed by Kato-Katz and 95.8% to 100% for *S. mansoni* diagnosed by formol-ether technique.

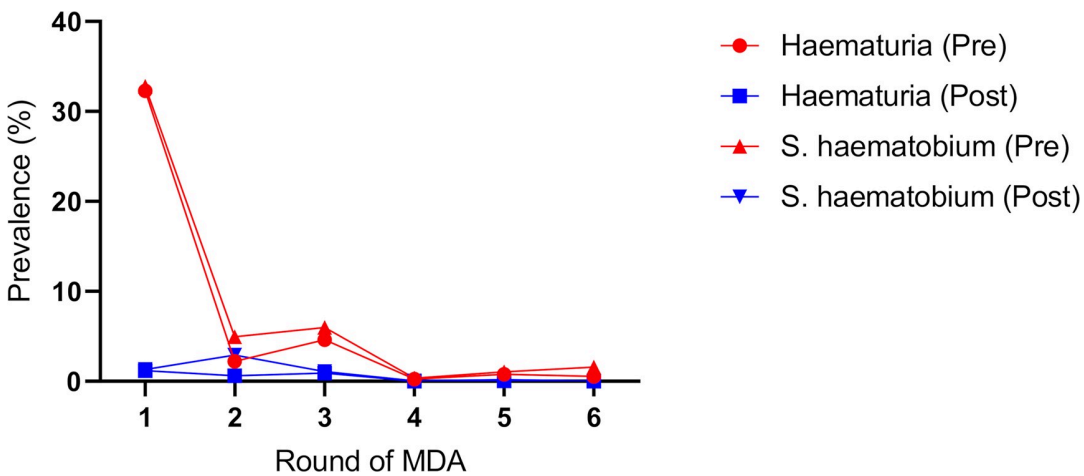

Fig 5. Decline in the prevalence of *S. haematobium* morbidity as measured by visible haematuria in the cohort of children.

https://doi.org/10.1371/journal.pntd.0008388.g005

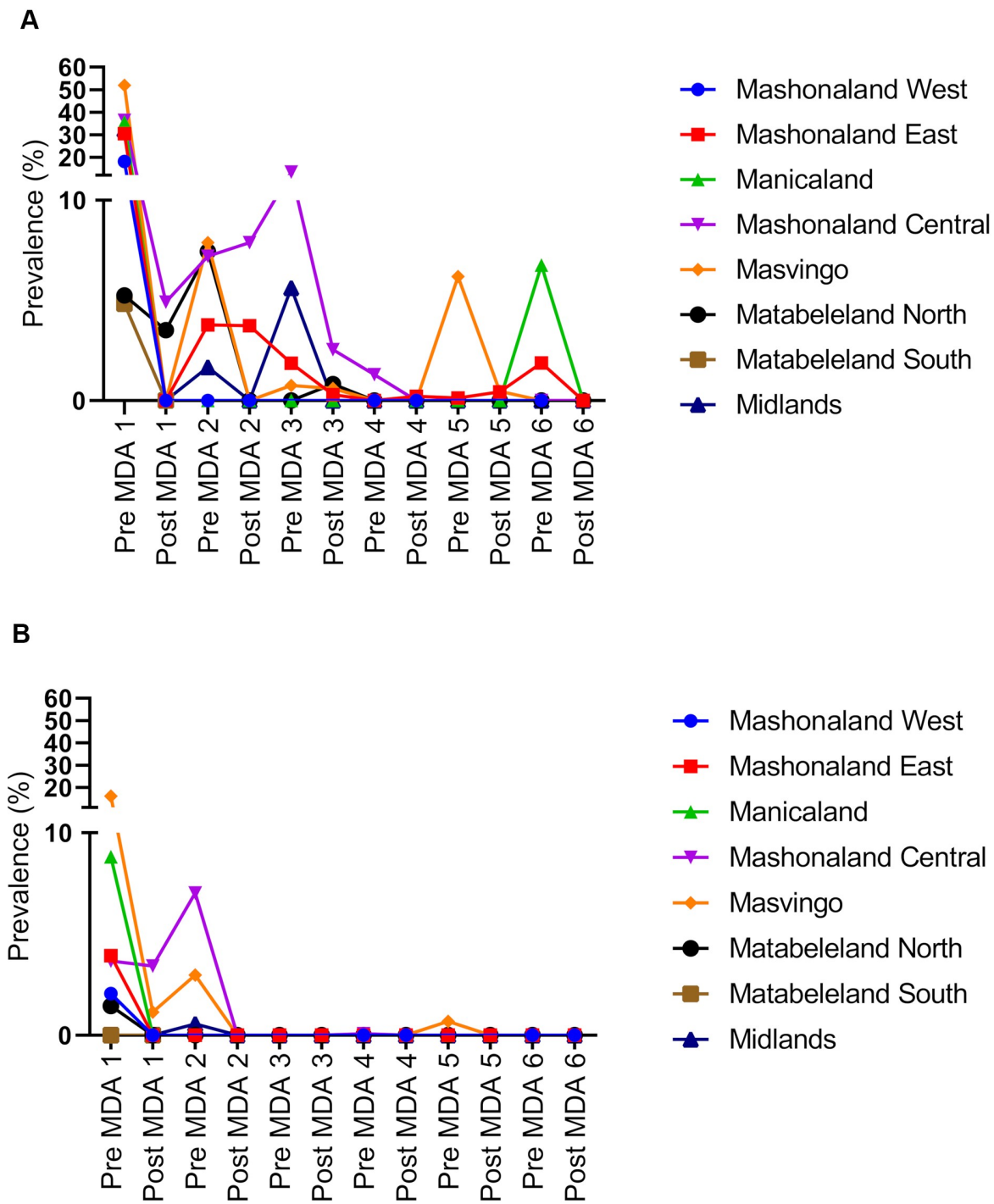

Fig 6. Decline in infection prevalence at province level during the MDAs. A. *S. haematobium*. **B.** S. *mansoni* as measured by Kato-Katz in the cohort of children.

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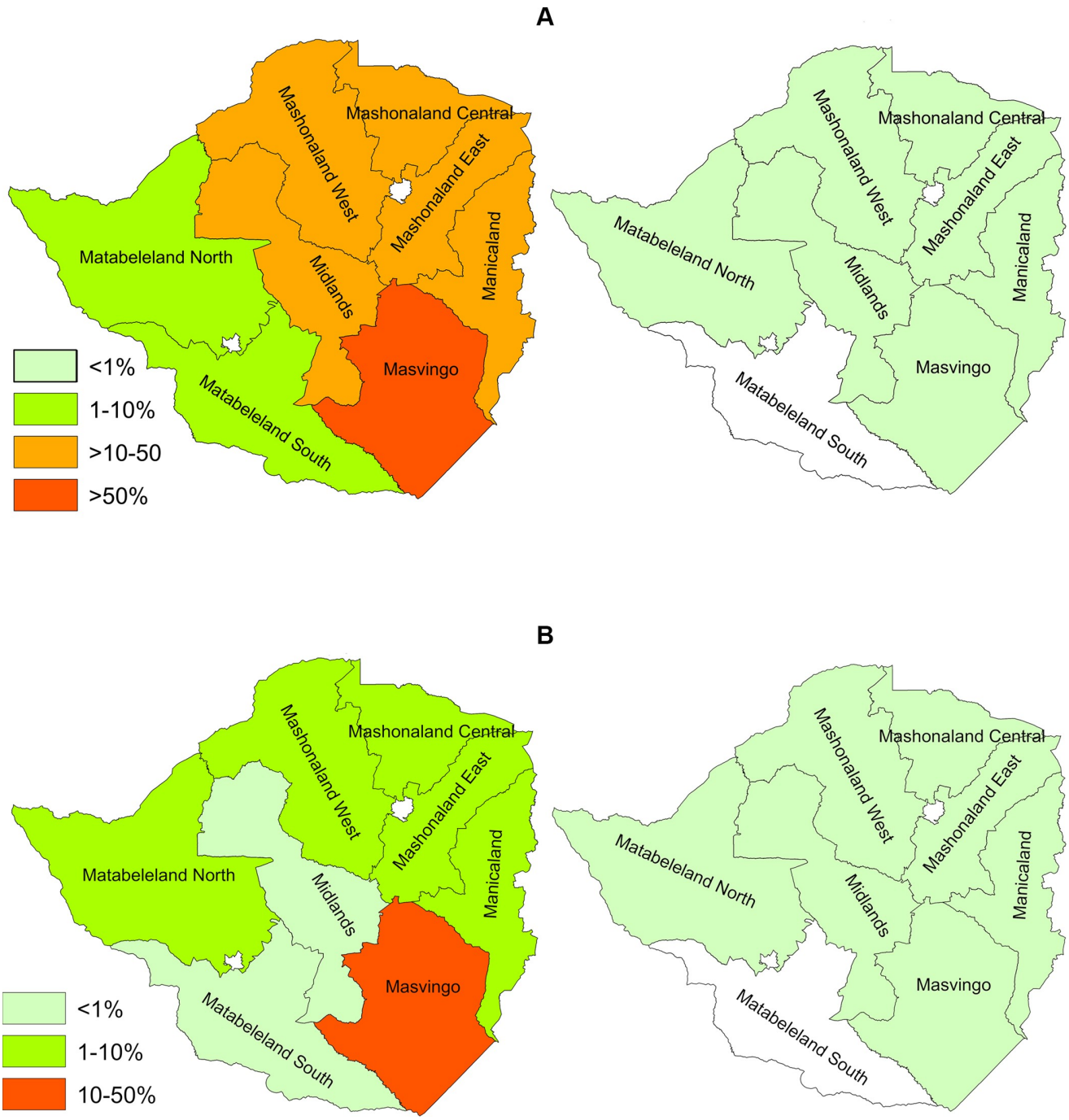

Fig 7. Changes in prevalence at national level in the cohort of children. A. *S. haematobium*. **B**. *S. mansoni*. The maps were generated using GIS raw data for the schools using ArcMap 10.1.

https://doi.org/10.1371/journal.pntd.0008388.g007

Treatment coverage

Mean coverage across all the provinces was lowest in MDA 1 at 48%, but this increased gradually peaking at 90.3% in the cohort during MDA 5 (Fig 8). While the mean national coverage

Table 2. Mean egg reduction rate (%).

MDA	*S. haematobium*	*S. mansoni* (Kato-Katz)	*S. mansoni* (Formol-Ether)
1	99.29	95.72	98.58
2	91.16	N/A	100
3	96.99	100	N/A
4	100	100	N/A
5	97.77	100	N/A
6	100	N/A	N/A

N/A represents MDA where the pre-treatment infection intensity was 0 eggs/ml or 0 eggs/mg.

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at MDA 6 was 82.3% there was heterogeneity between districts with coverage ranging from 73.5% (Mashonaland West) to 96.5% (Mashonaland Central) for MDA 6 (**S3 Fig**).

Discussion

The Uniting to Combat NTDs score card calculates Zimbabwe's efforts to combat NTDs in terms of average of coverage across the five diseases amenable to mass treatment (schistosomiasis, soil transmitted helminths (STH), river blindness, trachoma, elephantitis) as 12% but indicates that the coverage for schistosomiasis is 100% (see https://unitingtocombatntds.org/africa/zimbabwe/). However, these score card data do not indicate the effectiveness of the country's helminth control program in terms of reducing infection level or morbidity. This study presents the results from the analysis of the effect of annual praziquantel treatment on schistosome infection and morbidity in a cohort of primary school children during the national helminth control program in Zimbabwe. The study showed that upon sustained annual MDA using PZQ to treat schistosome infections and morbidity, both schistosome infection and morbidity have been significantly reduced in this cohort of children, meeting the WHO aim of reducing morbidity and infection in school-aged children [22].

Zimbabwe adopted a country strategy to treat all affected areas annually for 6 years. By surveying sentinel sites before the MDA and 6 weeks after the MDA every year, we have been able to demonstrate that following the third MDA, the prevalence of schistosomiasis in the cohort of school children in every province fell under the low-risk category deemed by the WHO i.e. prevalence of less than 10% [14][15] and remained there consistently throughout the rest of the programme.

Zimbabwe is one of the 41 African countries mentioned in the latest WHO Report on NTDs [23], as currently having preventive chemotherapy programs for schistosomiasis. Of these countries 28 (68.3%) which include Zimbabwe, were implementing preventative

Table 3. Mean cure rates (%).

MDA	*S. haematobium*	*S. mansoni (Kato-Katz)*	*S. mansoni* (Formol-Ether)
1	93.98	92.83	95.83
2	84.84	100.00	100.00
3	83.39	N/A	N/A
4	100.00	100.00	N/A
5	100.00	100.00	100.00
6	100.00	N/A	N/A

N/A represents MDA where the pre-treatment infection intensity was 0 eggs/ml or 0 eggs/mg.

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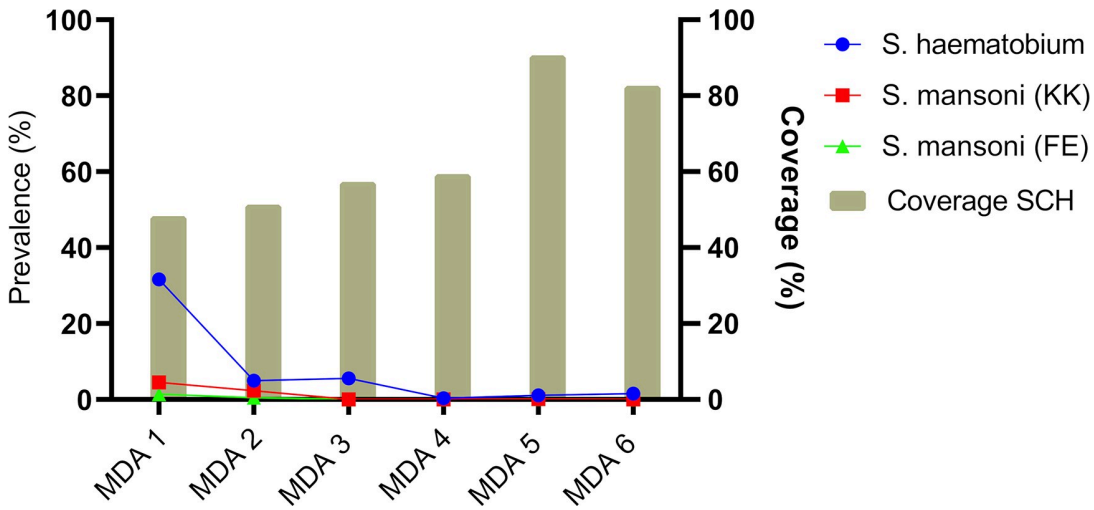

Fig 8. Relationship between treatment coverage rates and schistosome infection prevalence across the MDAs in the cohort of children.

https://doi.org/10.1371/journal.pntd.0008388.g008

chemotherapy programs in 2015. Of these only 15 had extended coverage to all endemic areas. In addition to this annual treatment across all levels of schistosome endemicity, the treatment coverage obtained each year generally increased from MDA 1 throughout the program. Maximum coverage reached for all school children targeted in Zimbabwe's helminth control program which included the cohort of children in this study, was 90%. This was above the recommended 75% coverage of school-aged children as detailed in the "2020 roadmap" set out by the WHO [9].

While there was an overall reduction in infection prevalence and intensity over the duration of the control program in the cohort of school children, there was some rebound in infection particularly over the first 3 years. This is unsurprising as there were no control efforts directed at breaking the transmission cycle. Studies in Kenya indicate that the reinfection can be sufficiently intense as to warrant more than an annual treatment [24]. In Zimbabwe, with increasing treatment coverage came lower re-infection rates culminating in the reduction of prevalence to 0% in this cohort of school children following the last MDA.

For both *S. mansoni* and *S. haematobium*, ERR were above 90%. Across the MDAs, average CR ranged from 83.4% to 100% for *S. haematobium* and 92.8% to 100% for *S. mansoni* diagnosed by Kato-Katz and 95.8% to 100% for *S. mansoni* diagnosed by formol-ether technique. Variations in treatment outcome have been previously reported and have prompted studies into trying to identify better measures of PZQ efficacy since parasite egg excretion is only a proxy for drug effects on adult worms [25]. Egg excretion can be confounded by various host or parasite factors, including facultative temporary cessation of excretion by the adult worms [25]. Variation in both CR and ERR measures could be attributable to parasite- or host-based factors including varying efficacy of PZQ in the killing of *S. haematobium* and *S. mansoni* in co-endemic areas [26], the intensity of the initial infection, host genetics, and the sensitivity of the diagnostic tests used to identify either species. For example, the urine filtration technique and Kato-Katz technique can miss low-level infections [27]. In areas with a particularly high prevalence of schistosomiasis, there is a greater chance of individuals being infected with immature schistosomes at the time of MDA treatment and therefore will not be completely cleared by one dose of praziquantel [28]. Furthermore, in areas of high transmission, high levels of reinfection can occur especially if the MDA occurs before the transmission period.

Treatment of only school children excluding adults and preschool children potentially maintains transmission. All of these factors may explain the observed persistence of infection post-treatment, such as that of Mashonaland Central in both *Schistosoma* species, for the first 3 MDAs in this study [29, 30].

The possibility of the parasites developing resistance to PZQ is always a present threat, with reports of low CR in certain regions [31, 32]. Indeed reduced efficacy of PZQ has been reported following multiple rounds of MDA in Uganda for *S. mansoni* [33]. To counteract these fears, investigations into multiple cases of laboratory and field PZQ resistance determined that the reduced efficacy of drug treatment due to resistance was rare [34]. Furthermore, a meta-analysis of PZQ CR and ERR we have conducted indicates that these have not changed significantly in the past 20 years. The reduction of infection at the sentinel sites to 0% and 0 egg counts for both schistosome species reflects that treatment remained efficacious throughout the MDA period. As Zimbabwe intensifies control efforts to move towards eliminating schistosomiasis as a public health problem, it will be important to collect parasite samples from infection hot spots to determine causes of persistent infection [35].

While Zimbabwe has made significant progress towards the control of schistosomiasis through this 6-year MDA program, more still needs to be done; first to maintain the low infections achieved in the primary school children who were the target of Zimbabwe's helminth control program represented by the cohort of children studied here and, second to include all populations at risk of infection i.e. adults and preschool children. The 2012 World Health Assembly resolution 65.21 advocates for elimination of schistosomiasis transmission in member states. This is an aspirational goal for Zimbabwe although in practise a difficult task. Annual MDA is not sustainable long-term for most endemic countries both for economic and logistical reasons in health systems with low budgets. It is also not effective on its own, at meeting the elimination aspiration of WHA 65.21. Thus, Zimbabwe, similar to all other schistosome endemic countries aiming to eliminate the disease, needs to strengthen concerted efforts to both reduce infection and morbidity, and also interrupt transmission. This must be based on integrated control approaches including intense PZQ treatment targeted at any hot spots of infection/transmission, snail control, treatment of infective water and improved access to safe water, sanitation and hygiene (WASH) [36]. The impact of such an integrated approach has been demonstrated in Egypt, where schistosomiasis is being tackled through targeted MDA and WASH improvements [37]. The lack of integrated comprehensive approaches to reduce both infection levels and transmission as well as the short-term timescale of MDA may explain the challenge experienced by other counties for example, Kenya [24] and Uganda [38], in improving the impact of MDA alone on infection levels/transmission. A comprehensive national impact assessment exercise for the national control program as is planned by Zimbabwe's Ministry of Health at the end of this initial phase of the country's helminth control program will be informative in shaping the next stages in the country's control of schistosomiasis.

We designed the study sampling framework based on the results of the national control survey which formed the basis of Zimbabwe's national control program [2]. Surveying all sentinel sites at every survey point would have given more information, but the perceived benefit from this did not justify the associated expense. In the national survey, 50 children per school were sampled, whereas in this study, in the majority of schools we sampled at least 200 children. Sample sizes lower than 200 were reflective of the enrolment numbers of children of the appropriate age group at the school. The follow-up of 75% of the children over the 6 years reflects predominantly children who moved school to a different area and could not be traced. Children remained eligible for follow-up if they moved to the local catchment area senior school

i.e. within the same district and often served by the same river system to avoid following children who moved to areas of different schistosome transmission dynamics.

The data were presented summarily at province level; however, as control efforts strengthen in the move towards elimination, more refined mapping at district level, therefore more schools, will be more informative. For infection diagnosis, we used the parasitological examination of urine and stool samples and this showed the reduction of egg counts to 0 for both species of schistosome infections. Although the parasitological examination of urine and stool is the currently recommend diagnostic for schistosomiasis, we and others have previously shown that the approach is less sensitive when infection levels are low [27] and therefore more accurate diagnostic tools are needed to monitor progress of control efforts. Alternative diagnostic approaches including PCR [39] and parasite antigens [40–43] have been used by others, albeit not routinely in national MDA programs where there may be challenges with diagnostic accuracy [44]. Although beyond the scope of this study, information on the transmission dynamics in the local infective water bodies which the children frequented would have added valuable information on the impact of treatment on the force of infection.

On a schistosome elimination agenda, there is need to extend treatment to cover the whole community, including preschool aged children (aged 5 years and below) as well as adults. Indeed the guidelines from WHO indicate that preventative chemotherapy should be targeted at all groups at risk of infection in highly endemic communities [45]. Quantitative work has shown that integrated community-wide treatment for schistosomiasis and soil-transmitted helminths can be highly cost effective even in areas of low endemicity for either schistosomiasis or STH [46]. It is also important to be cognizant of the fact the current WHO guidelines were not designed with the goal of elimination so current control strategies will need to be adapted to deliver elimination while new or better tools for diagnosis and verification of cessation of transmission will need to be developed. Indeed, the WHO already acknowledges the need for flexibility in control approaches for different counties in the roadmap for controlling and eliminating NTDs [9]. Thus Zimbabwe's approach of annual treatments regardless of the schistosomiasis endemicity level at the operational region may have been more effective. Zimbabwe's last MDA was in 2017 and the country is now evaluating the impact of the control program to inform the next steps in the country's helminth control action plan.

In conclusion, based on the survey of schistosome infection and *S. haematobium* morbidity in a cohort of school children in sentinel sites across the country, Zimbabwe's helminth control program significantly reduced schistosome infection and morbidity levels in this cohort of school-age children to levels where elimination is a possible goal. If extrapolated to national level, the control program moved the country from a moderate to low endemicity levels as per WHO classification for schistosomiasis [14]. The findings of this study will inform the design of the country's integrated control strategy towards elimination following a national assessment of the impact of the national helminth control program at all previously surveyed sites.

Supporting information

S1 Fig. The 35 sentinel sites chosen to represent districts (and thus provinces) in the country. The districts are colour coded to represent baseline schistosome infection prevalence classified into low infection (coloured grey), moderate infection (coloured blue) and heavy infection (coloured red) and indicating the number of surveys conducted per sentinel site. Mashonaland and Matabeleland Provinces are abbreviated thus: Mash West = Mashonaland West, Mash East = Mashonaland East, Mash C = Mashonaland Central, Mat N = Matabeleland North, Mat S = Matabeleland South.
(DOCX)

S2 Fig. Combined prevalence of *S. haematobium* and *S. mansoni* in the cohort of Zimbabwean school children (a) before year 2012, and (b) after year 2017 Mass Drug Administration with Praziquantel. Maps were generated using the primary raw data and plotted using ArcMap 10.1.
(PDF)

S3 Fig. Summary % coverage of schistosome treatment at provincial level in the school children cohort in the last MDA in 2017.
(TIF)

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PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Investigating a strategy for quantifying schistosome infection levels in preschool-aged children using prevalence data from school-aged children

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Abstract

In 2012, the World Health Organisation (WHO) set out a roadmap for eliminating schistosomiasis as a public health problem by 2025. To achieve this target, preschool-aged children (PSAC; aged 6 years and below) will need to be included in schistosomiasis treatment programmes. As the global community discusses the tools and approaches for treating this group, one of the main questions that remains unanswered is how to quantify infection in this age group to inform treatment strategies. The aim of this study was thus to determine whether a relationship exists between levels of schistosome infection in PSAC and school-aged children (SAC), that can be used to determine unknown schistosome infection prevalence levels in PSAC. A systematic search of publications reporting schistosomiasis prevalence in African PSAC and SAC was conducted. The search strategy was formulated using the PRISMA guidelines and SPIDER search strategy tool. The published data was subjected to regression analysis to determine if a relationship exists between infection levels in PSAC and SAC. The interaction between SAC and community treatment history was also entered in the regression model to determine if treatment history significantly affected the relationship between PSAC and SAC prevalence. The results showed that a significant positive relationship exists between infection prevalence levels in PSAC and SAC for *Schistosoma mansoni* ($r = 0.812$, df (88, 1), $p = <0.0001$) *and S. haematobium* ($r = 0.786$, df (53, 1), $p = <0.0001$). The relationship was still significant after allowing for diagnostic method, treatment history, and the African sub-region where the study was conducted (*S. mansoni*: $F = 25.63$, df (88, 9), $p = <0.0001$; *S. haematobium*: $F = 10.20$, df (53, 10), $p = <0.0001$). Using the regression equation for PSAC and SAC prevalence, over 90% of the PSAC prevalence studies were placed in the correct WHO classifications category based on the SAC levels, regardless of treatment history. The study indicated that schistosome prevalence in SAC can be extended as a proxy for infection levels in PSAC, extending on its current use in the

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adult population. SAC prevalence data could identify where there is a need to accelerate and facilitate the treatment of PSAC for schistosomiasis in Africa.

Author summary

Preschool-aged children (PSAC), i.e. aged $\leq$ 6 years, are not included in preventative chemotherapy programmes, currently advocated by the WHO for the treatment of schistosomiasis. This is due to the lack of a paediatric formulation of the drug of choice, praziquantel, and the current guidelines which requires diagnosis before treatment. As the global community prepares for the deployment of a new paediatric formulation of praziquantel, there is a need to find a strategy to quantify infection in this age group. In schistosome endemic areas, infection levels in school-aged children (SAC) are already used to inform infection levels in the community. Thus, we investigated the relationship between SAC and PSAC schistosome prevalence levels within the same community, to determine if data from SAC could be used to predict infection levels in PSAC. Our results show that PSAC prevalence levels are significantly correlated with SAC prevalence levels. Our findings are applicable to communities that have received preventative chemotherapy in SAC or in SAC and adults, as well as those that have not received any. Our study indicates that it is possible to extrapolate PSAC prevalence levels from SAC prevalence, and to make a treatment decision on that basis.

Introduction

Schistosomiasis is a widespread parasitic disease found in tropical and subtropical areas [1]. The World Health Organization (WHO) estimated that at least 206 million people worldwide required treatment for schistosomiasis in 2016, with at least 91% living in Africa [2]. The most widely used treatment for schistosomiasis is the antihelminthic drug of choice, praziquantel (PZQ), which is both safe and efficacious against adult worms [3]. Currently, schistosomiasis is controlled through preventative chemotherapy, targeting school-aged children (SAC) who are treated with PZQ through mass drug administration (MDA). The frequency of treatment follows guidelines from the WHO, which are based on the schistosome endemicity of the area rather than individual infection status [4]. The endemicity of the area is determined by quantifying schistosome infection prevalence in SAC, following sampling of a group of the children. The reason SAC are used for determining community schistosome endemicity and are the primary target of MDA is that they have been shown to have the highest prevalence of infection and are easily accessible in schools [5, 6].

As of November 2018, 29 of the 41 African countries requiring preventive chemotherapy have implemented MDA preventative chemotherapy in SAC; approximately 75 million children have already received treatment [7]. However, none of these programmes include preschool-aged children (PSAC; aged 6 years and below). In endemic areas, children can be infected as early as 1 year old, and the burden of infection as well as disease morbidity increases with age [8, 9]. Several authors have highlighted the gap in treatment strategies, created by the exclusion of PSAC (reviewed by Stothard *et al.* 2013) [10]. This creates a health inequity for approximately 50 million African PSAC exposed to schistosomiasis [11].

In order to eliminate schistosomiasis as a public health problem by 2025, based on the WHO roadmap on neglected tropical diseases (NTDs) [12], there is a need to include PSAC in

treatment programmes. The WHO have responded by recommending the treatment of PSAC [13], and also calling for the development of a paediatric formulation of PZQ, suitable for treating PSAC. There is now a paediatric formulation of PZQ under development (currently undergoing Phase III clinical trials) targeting children aged 3 months to 6 years. Thus, in preparation for inclusion of PSAC in treatment programmes, either as part of MDA or treatment upon diagnosis, there is a need to develop a strategy for quantifying infection in this age group.

Unlike SAC who are readily accessible in schools, PSAC are not often as accessible, and conducting mapping exercises to quantify their infection levels can be challenging and costly. Therefore, we aimed to determine if SAC infection prevalence data could be used to determine infection prevalence in PSAC. In this study, we investigated the relationship between schistosome prevalence levels in SAC and PSAC, to determine if SAC infection levels in Africa can be used as a predictor of the prevalence levels in PSAC from the same community [13, 14].

Methods

This study focused on published manuscripts reporting schistosome infection prevalence, derived from parasitological detection of schistosome eggs excreted in urine or stool. A systematic search of publications that reported schistosomiasis prevalence in PSAC and SAC in Africa was carried out. The search strategy was formulated using the PRISMA guideline (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) [15], and the SPIDER search strategy tool (Sample, Phenomenon of Interest, Design, Evaluation, Research type) [16] in order to formulate a search question (see Table 1).

Published studies were identified using three online databases: PubMed (conception to 30th October 2018), Embase (1980–2018, week 44) and Web of Science (1983 – 30th October 2018). The results were filtered to show only those published in English.

Criteria for inclusion and exclusion of studies

Results from the literature searches were consolidated and extracted using EndNote referencing software (X8.01). Endnote and a Python script were used to match and remove duplicate titles.

Eligibility for inclusion was based on the following criteria: i) the survey was carried out in Africa, ii) it was published/written in English, iii) it contained prevalence data for PSAC, SAC

Table 1. Search terms created using the SPIDER search strategy.

Sample	Children (preschool and school age)
Phenomenon of interest	Schistosomiaisis infection, either *S. haematobium* or *S. mansoni*
Design	Cross-section, survey
Evaluation	Egg count in urine or stool
Research type	Not included in search criteria but reviews and meta-analysis were removed during screening
Terms used in search	
S	Children OR Preschool OR Pre - school OR Infant OR Infants OR PSAC OR SAC
	AND
PI	Schisto* OR Bilharzia
	AND
D	Cross section OR Cross-section OR Cross-sectional OR Survey OR Prevalence
	AND
E	Urine OR Stool OR Katz OR egg OR eggs
R	N/A

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or both, iv) the sample size was clearly stated, v) the sample size for each age group was above 10 people, vi) parasites identified were either *S. mansoni* or *S. haematobium*, vii) diagnosis was via egg count in stool or urine samples, viii) the age ranges were clearly defined, and ix) PSAC age range was between 0 up to 6 years and SAC age range was between 6–16 years.

The studies were further assessed using the following criteria: recruitment of participants was random and non-biased, and sample collection and diagnostics was clearly explained and carried out according to standard protocols [6]. Any studies not meeting these criteria were excluded from the analyses. Data were entered based on prevalence of schistosomiasis per water source/village, therefore one citation could yield multiple datasets.

Quality assessment

The quality of each study included in the analysis was assessed using a graded scale. Each study was scored either 1 (yes) or 0 (no) against multiple criteria. There were 7 criteria–i) population sampling methods described; ii) results stratified by gender; iii) a description of laboratory diagnostics included; iv) multiple samples taken on different days; v) multiple tests of a single sample carried out (*S. mansoni* only); vi) water source and/or the sanitary conditions described; and vii) statistical analysis described in the methods. The scores were added together and the overall score per article graded as follows: high 6–7, medium 3–5, and low 0–2 for *S. mansoni*, and for *S. haematobium*, high 5–6, medium 3–4 and low 0–2. If any of the studies scored low on quality assessment, the analysis was conducted with and without the study, to determine if it had an effect on the overall results.

Study variables

Study variables extracted from the publications were: sample size, age range, egg detection method used for schistosome diagnosis, schistosome treatment history of the study communities, and the antihelminthic drug used for the treatment. The studies used different age ranges for defining PSAC and SAC. For this study PSAC was defined as ages 0–6 years and SAC were defined as 6 years up to 16 years of age. Techniques for detecting eggs in stool for *S. mansoni* diagnosis were i) formol-ether sedimentation [17], ii) the Bell Method [18], and iii) Kato-Katz thick smear [19]. For *S. haematobium*, egg techniques reported were urine sedimentation and filtration methods [20]. Studies were included in the analysis providing the paired PSAC and SAC infection were diagnosed using the same technique. History of schistosome treatment of the community or the study participants was recorded and included in the statistical analyses; this information was available at a community/group level but not individual participant level. Four categories of treatment history were recorded; 1) No treatment taken place prior to study (i.e. no recorded mass treatment in the past 50 years), 2) Treatment currently underway, 3) Treatment has been recorded within the past 5 years, and 4) No information available about treatment history. If the study did not state whether the community had undergone previous MDA, the WHO Preventative Chemotherapy Treatment (PCT) databank was used to assess whether treatment strategies were in place in this country or had taken place in the past 5 years [21].

Data extraction

All eligible articles were read in full. The following information was extracted and tabulated (Microsoft Excel); first author name, year of publication, dates of survey, country, African sub-region, sample size, age range, male to female ratio, overall prevalence of infection, infection intensity, male and female infection prevalence, standard deviation, standard error of the

mean and 95% confidence intervals, previous schistosome treatment history and method used for collecting the eggs for schistosomiasis diagnosis.

If the data were presented in smaller age ranges, for example, one of the studies specified groups were 6–10 years and 10–16 years, the sample sizes were added together, and from this combined group an average prevalence was calculated. When the age groups exceeded the limits defined by this study e.g. above 6 years old for PSAC or above 16 years old for SAC, the study was removed from the analysis. Furthermore, if the SAC group was partitioned into two, and the older group exceeded the upper limit of the grouping, only the younger group within the SAC was included. Where the published data were presented in graphs, Data-Thief software (Data Thief III), a highly accurate data extraction tool, was used to extract the exact numerical data [22].

Two categories of data were identified, Category 1 in which both PSAC and SAC data come from the same study, and Category 2 in which the data were from two separate studies. Category 2 studies were matched using the following additional criteria: the surveys were carried out within one year of each other, the population used the same water source, and the parasitology diagnostic techniques were the same.

Statistical analysis

Data analysis was carried out using MINITAB (version 18). As the infection data for both SAC and PSAC were provided as prevalences (recorded as percentage data), both variables were transformed using the arcsine square root transformation to satisfy the assumptions for parametric tests, and to avoid resultant negative prevalence values post-transformation [23]. We determined whether or not data satisfied the parametric assumptions through analysing the residuals (e.g. normality plots and histogram). We also validated the regression model by analysing the goodness of fit of the regression and analysing whether the regression residuals are random.

To determine the relationship between PSAC and SAC, Pearson's correlation and linear regression without any confounding factors were used. Thereafter, the relationship was analysed through linear regression, allowing for the effect of confounding categorical variables: the African sub-region where the study was conducted (North, South, East, West and Central (see https://unstats.un.org/unsd/methodology/m49/), diagnostic technique (*S. mansoni*–Kato katz, Formol-ether or Bell, and *S. haematobium*–Filtration or sedimentation), and history of schistosomiasis treatment (No previous treatment in population, treatment within the past 5 years, treatment currently underway and history not given). Also included in the model was the interaction between SAC infection prevalence and treatment history, to determine if the relationship between the two prevalences differed according to the treatment history of the study population.

The relationship between PSAC and SAC was tested for (after allowing for the effects of the above mentioned confounding variables), using adjusted sums of squares to calculate the *F*-value. Adherence to the assumptions of parametric tests was confirmed by a series of residuals plots including histogram plots, residual vs. fits and residuals vs. plots. The significance value was $p < 0.05$.

The goodness of fit of the model to the data was recorded as the *r*-squared value, and the regression coefficient's 95% confidence intervals were calculated using the equation:

95% CI = $b \pm tc_{(n-2)}$ x SE

Where;

b = regression coefficient

tc = Critical T = T value at 0.05 for n-2 degrees of freedom

n = study sample size.

SE = standard error of the coefficient

The 95% prediction band of the regression line were generated and plotted in GraphPad (Version 8). Statistical analyses were conducted with and without the data from the studies scoring low on the quality assessment (see S3 Table and S4 Table). This did not affect the result, and so the studies were included in the analysis.

Results

Literature search

The selection process is presented in Fig 1. Literature searches of online databases returned 3515 studies. 1263 duplications were identified and removed, leaving 2252 unique studies. After the screening and full text read, 68 studies and 143 datasets (89 for *S. mansoni* and 54 for *S. haematobium*) were used in the analysis.

Eligible studies

In total, 23 African countries had prevalence data which could be used in the analysis (Table 2) and these were spread across the continent (Fig 2).

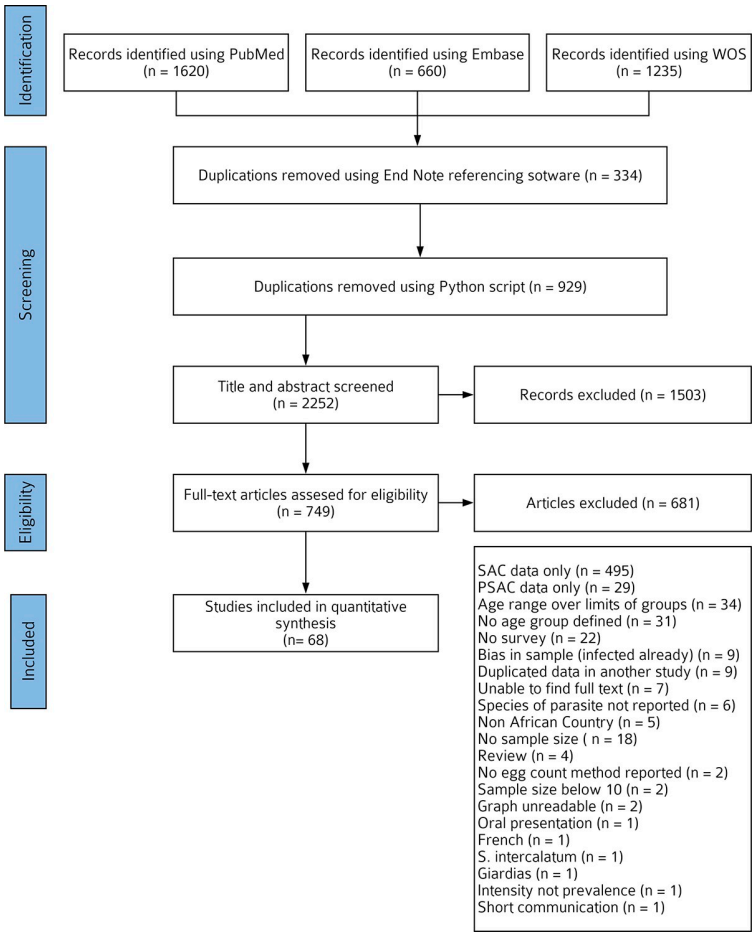

Fig 1. Flowchart for search and selection of included studies.

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Table 2. Publications and data sets included in analysis, broken down by country of origin.

	S. mansoni		*S. haematobium*	
Country	**Publications**	**Data sets**	**Publications**	**Data sets**
Botswana	0	0	1	1
Burundi	1	1	0	0
Cameroon	0	0	2	5
Cote d'Ivoire	4	7	5	6
Egypt	5	8	2	3
Ethiopia	4	8	1	1
The Gambia	0	0	1	1
Ghana	0	0	3	3
Kenya	5	9	0	0
Liberia	1	1	1	1
Malawi	1	1	0	0
Mali	1	1	1	1
Niger	1	1	3	3
Nigeria	1	1	7	7
Senegal	2	2	2	2
Sierra Leone	2	7	0	0
Somalia	0	0	1	3
South Africa	1	1	1	1
Sudan	1	5	2	6
Tanzania	1	1	3	2
Uganda	4	5	0	0
Zaire (DRC)	1	29	0	0
Zimbabwe	1	1	4	8

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Schistosoma mansoni extracted data

There were 89 paired data points for *S. mansoni* extracted from 40 manuscripts with surveys between 1974 and 2018. S1 Table, shows characteristics of all studies used in the meta-analysis for *S. mansoni* infection in PSAC and SAC. Sample sizes ranged from 11 to 1122 in PSAC (mean = 145, median = 68). Sample sizes in SAC ranged from 12 to 2856 (mean = 333, median = 107). The prevalence of infection ranged from 0–100% in PSAC (mean = 25%, median = 15%, the interquartile range (IQR) = 33.1, standard deviation (SD) = 25.8) and 2.5–100% in SAC, (mean = 50%, median = 47%, IQR = 58.6, SD = 30.3).

For infection detection, the Kato-Katz technique was used in 86 of the 89 (96.6%) investigations, 2 (2.2%) used the formol-ether technique, and 1 (1.1%) used the Bell Method for egg detection in stool. Out of 89 data points, no treatment had previously taken place in 72 (80.9%) communities, 13 (14.6%) of the communities reported treatment currently underway, 1 (1.1%) study reported one round of MDA 2 years previously but no further treatment had taken place, and 2 communities (2.2%) did not report whether control strategies had taken place or not. For the *S. mansoni* analysis, 77 were Category 1 (data was from the same study) and 11 were from Category 2 (data was from two separate studies and adhere to the additional inclusion criteria).

Schistosoma haematobium extracted data

For *S. haematobium*, there were 54 paired data points from 39 manuscripts, and surveys occurred between 1962 and 2016. S2 Table shows characteristics of all studies that investigated

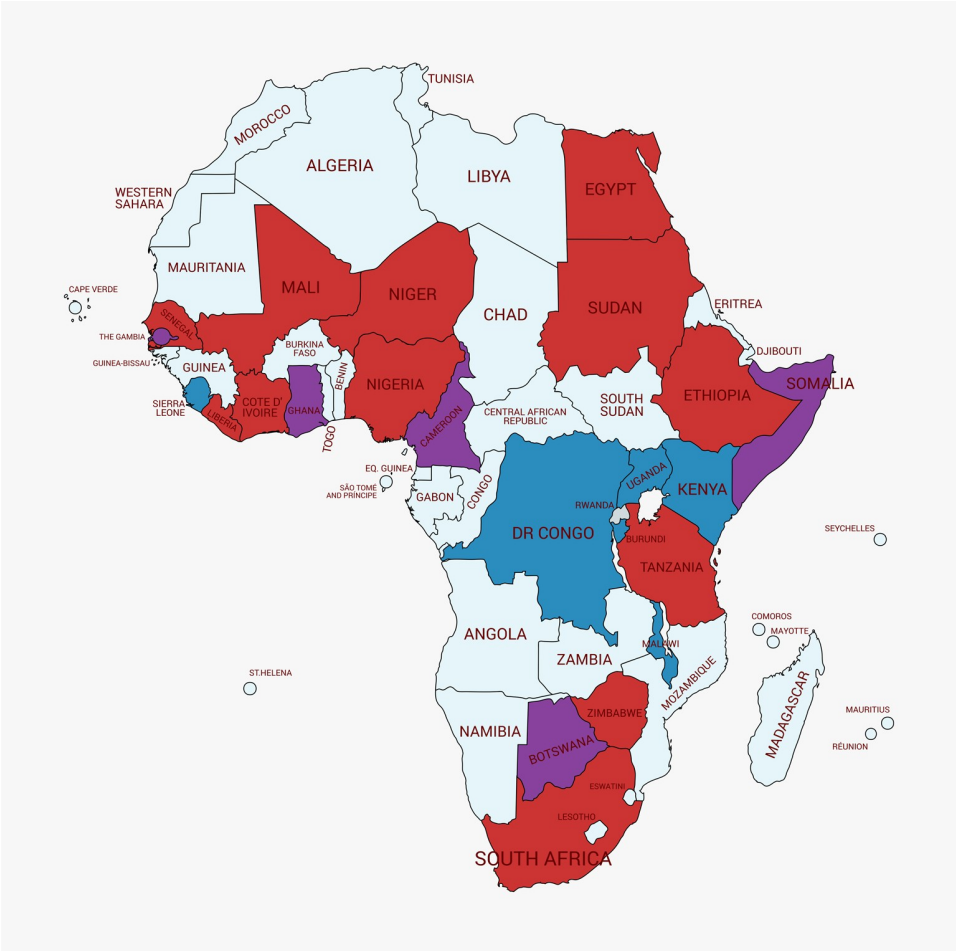

Fig 2. Map of African countries included in the analysis. The map shows the countries where the data came from, partitioned by schistosome species, red = both *S. mansoni* and *S. haematobium* data present, blue = *S. mansoni* only, purple = *S. haematobium* only and pale blue = no data used from these countries. The map was made using the online MAPCHART software package (https://mapchart.net/africa.html).

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S. haematobium prevalence in PSAC and SAC. Sample sizes ranged from 11 to 1018 in PSAC (mean = 141, median = 78). Sample sizes of SAC ranged from 21 and 4326 (mean = 557, median = 215). The prevalence of infection ranged from 0–88% in PSAC (mean = 26.2%, median = 19.9%, IQR = 29.5, SD = 23.84) and 0–99% in SAC (mean = 51.5%, median = 54.2%, IQR = 36.7, SD = 27.9). Two diagnostic methods were included in the analysis, the filtration technique [20] was used in 36 of the 54 investigations, and the sedimentation technique was used in the remaining 18 studies.

No treatment had taken place prior to the study in 41 out of 54 (75.9%) of the data sets analysed, 8 out of 54 (14.8%) reported treatment currently underway, 1 (1.9%) study reported treatment of previously positive SAC with Niridazole, about 5 years previously, and 4 (7.4%) had no information on previous treatment status. For the *S. haematobium* analysis, 51 were Category 1, and 3 were from Category 2.

The sample sizes for the different independent variables are given in Table 3. Age range for PSAC across the published studies for both schistosome species was <1 up to 6 years, and for SAC, age range was 6–16 years. While for all studies PSAC sample sizes were lower than SAC

Table 3. Summary of sample sizes for categories.

Variable	Categories	*Schistosoma mansoni*	*Schistosoma haematobium*
African Region	North	13	9
	South	1	2
	East	26	14
	West	20	24
	Central	29	5
Treatment History	No previous treatment	73	41
	Currently undergoing treatment	13	8
	MDA previously conducted in the area	1	1
	Treatment history information not provided	2	4
Diagnostic Method	Urine Filtration	N/A	36
	Sedimentation	N/A	18
	Kato Katz	87	N/A
	Formol Ether Technique	1	N/A
	Bell	1	N/A

N/A = does not apply

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sample sizes, the sample sizes recorded in the published studies for both species were correlated in PSAC and SAC i.e. those studies with low sample sizes for PSAC also had low sample sizes for SAC and the converse was true. Therefore, the regression analysis was weighted with the square root of the PSAC sample size.

Relationship in schistosome infection prevalence between preschool-aged children (PSAC) and school-aged children (SAC)

The Pearson correlation showed a linear relationship between PSAC infection prevalences and SAC infection prevalence in the same communities for both *S.mansoni* ($r = 0.812$, $df = 1$, $p = <0.0001$) and *S. haematobium* ($r = 0.786$, $df = 1$, $p = <0.0001$). This was subsequently analysed through linear regression.

The simple regression analysis was carried out without the effect of confounding variables, and was weighted by the square root of PSAC sample size. This showed a significant relationship between *S. mansoni* PSAC and SAC prevalences, with 65.9% of the variation in *S. mansoni* PSAC prevalence being explained by the model with SAC infection prevalence alone (95% CI = 54.7–77.1). Similarly, the regression analysis showed a significant linear relationship between *S. haematobium* PSAC and SAC prevalences with 61.8% of the variation in *S. haematobium* PSAC prevalence being explained by the model with SAC infection prevalence alone (95% CI = 46.4–77.2). The simple relationship between PSAC and SAC prevalences is shown in Fig 3A and 3B, and the results of the regression analysis are summarised in Table 4 (*S. mansoni*) and Table 5 (*S. haematobium*).

The WHO provides guidelines [4] for the optimal frequency of schistosome treatment of SAC, by assessing the prevalence of infection or visible haematuria in a community. We therefore coded the infection prevalence in PSAC using the guidelines for SAC (low, moderate and high) to indicate levels of infection in PSAC, which would be captured under those categories (see Fig 4). It can be seen that when SAC prevalence levels are low, there is a low infection prevalence in PSAC, whereas when infection levels are high in SAC, levels in PSAC are moderate to high. There are two instances where PSAC infection levels are higher than in SAC, only one published report for each schistosome species, and the fitted line in Fig 4 predicts that a 100% infection prevalence is reached earlier in SAC than PSAC.

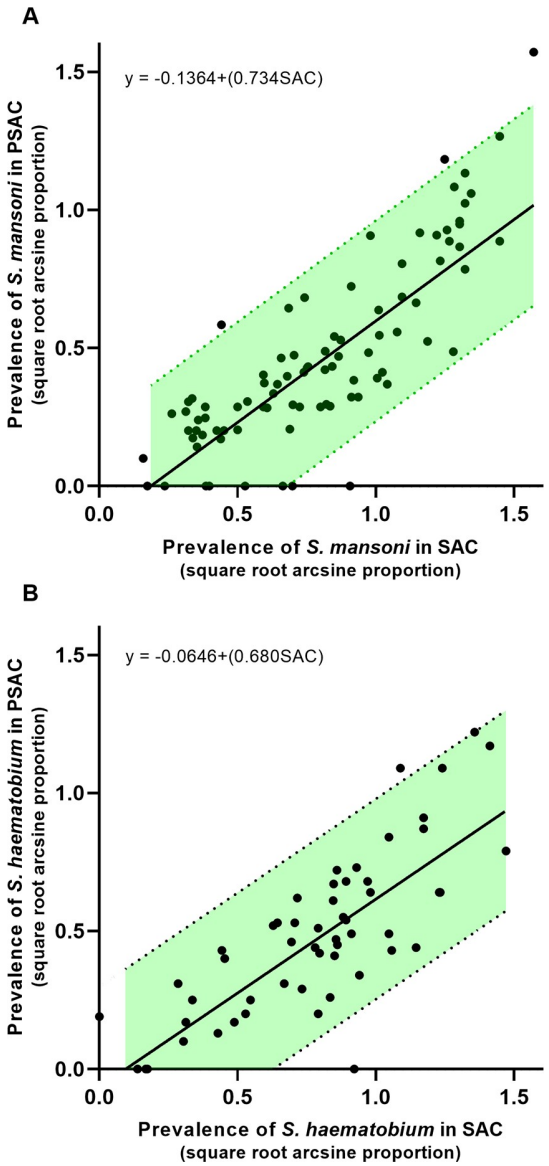


Fig 3. Scatterplots of PSAC vs SAC prevalence with 95% confidence band of the regression line for A) *S. mansoni* and B) *S. haematobium*. Fitted line is from the linear regression analysis.

https://doi.org/10.1371/journal.pntd.0008650.g003

The regression predicts PSAC prevalences for the WHO categories based on the SAC prevalences. For example, when SAC levels are low in *S. mansoni*, i.e. 0–9%, the PSAC levels are interpolated to be <1% (Fig 4A). Therefore based on the prediction, we determined the proportion of PSAC studies wrongly classified into a lower infection level, based on the SAC prevalences. Being, misclassified into a lower level is important from a public health perspective, as it means infection levels in PSAC will be underestimated.

For *S. mansoni*, the PSAC infection was underestimated in 4/89 study populations (4.5%). One study population (out of 4) had a PSAC prevalence that was classified in the low WHO category based on its SAC prevalence when it had a PSAC prevalence greater than 1% and therefore, should have been in the moderate category. This study reported the same prevalence 6.7% for both SAC and PSAC. Additionally, 3/42 (7.1%) study populations were classified as

Table 4. Analysis of variance and coefficients—*S. mansoni*, *basic regression weighted by square root of* PSAC*n*.

Source			df	F-value	P- value
Regression			88 (1)	168.17	<0.001
Prevalence in SAC			88 (1)	168.17	<0.001
Coefficients	**Coef**	**SE Coef**	**95% CI Lower**	**95% CI Upper**	**P- value**
Constant	-0.136	0.049	-0.265	-0.007	0.006
Prevalence in SAC	0.734	0.056	0.623	0.845	<0.001

Abbreviations: n–sample size, PSAC–preschool age children, SAC–School age children-square root arcsine transformed, df- degrees of freedom, Coef–coefficient, SE–Standard error.

Mathematical equation for the model: PSAC prevalence = A + bSAC prevalence

Where A is the constant coefficient and b is the Prevalence in SAC coefficient

Therefore: PSAC prevalence = -0.136 + 0.734 x SAC prevalence

https://doi.org/10.1371/journal.pntd.0008650.t004

moderate when their PSAC prevalences would have put them in high infection level. Of these 3 populations, 1 was undergoing treatment. For *S. haematobium*, in total 4/54 (7.4%) PSAC study populations were underestimated. One of the study populations (out of 4) had a PSAC prevalence that was classified in the low infection WHO category when it should have been in the moderate category. This study had a PSAC prevalence of 9% which was greater than the SAC prevalence of 7.9%. Further, 4/18 (22.2%) study populations were classified as moderate when their PSAC prevalences would have put them in high infection level. Of these 4 populations, 1 was undergoing treatment.

Effect of treatment history on the relationship between infection prevalence between preschool-aged children (PSAC) and school age children (SAC)

We conducted a regression analysis, allowing for the African sub-region where the study was conducted for both schistosome species, as well as the parasitology diagnostic method for *S. haematobium* only as there was not enough data in the diagnostic categories in the *S.mansoni* analysis to give a meaningful investigation. This was to determine if the relationship between PSAC and SAC prevalences varied significantly with the treatment history of the study community. The African sub-region was included in the analysis, as earlier descriptive statistics had indicated that there was heterogeneity in infection prevalences reported by studies from the different countries in Africa; this is confirmed by the significant effect of this variable on

Table 5. Analysis of variance and coefficients—*S. haematobium*, *basic regression weighted by square root of* PSAC*n*.

Source			df	F-value	P- value
Regression			54 (1)	84.28	<0.001
Prevalence in SAC			54 (1)	84.28	<0.001
Coefficients	**Coef**	**SE Coef**	**95% CI Lower**	**95% CI Upper**	**P- value**
Constant	-0.065	0.062	-0.189	0.059	0.300
Prevalence in SAC	0.680	0.074	0.531	0.828	<0.001

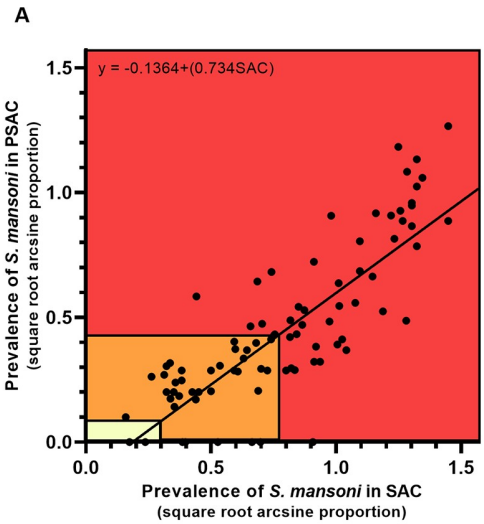
Abbreviations: n–sample size, PSAC–preschool age children, SAC–School age children-square root arcsine transformed, df- degrees of freedom, Coef–coefficient, SE–Standard error.

Mathematical equation for the model: PSAC prevalence = A + bSAC prevalence

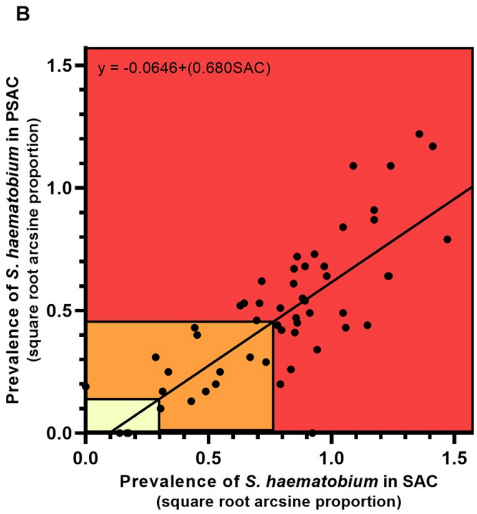
Where A is the constant coefficient and b is the Prevalence in SAC coefficient

Therefore: PSAC prevalence = -0.065 + 0.680 x SAC prevalence

https://doi.org/10.1371/journal.pntd.0008650.t005



SAC - prevalence (%)	SAC - Arcsine transformation	PSAC - prevalence (%)	PSAC - Arcsine transformation
0 - 9%	0 - 0.31	< 1	0 - 0.091
10 - 49%	0.32 - 0.78	1-18%	0.099 - 0.436
50 - 100%	0.79 - 1.57	≥19	0.444 - 1.016



SAC prevalence (%)	Arcsine transformation	PSAC - prevalence (%)	PSAC using equation
0 - 9	0 - 0.31	≤ 2	0 - 0.146
10 - 49	0.32 - 0.78	3 -20	0.153 - 0.466
50 - 100	0.79 - 1.57	≥ 21	0.473 - 1.003

Fig 4. Scatterplots of PSAC vs SAC prevalence demarcated into the WHO prevalence classes for A) *S. mansoni* and B) *S. haematobium*. The classes are shown according to SAC prevalence levels showing the corresponding PSAC level on the Y-axis. Red = High, Orange = Moderate, Yellow = Low. The fitted line is from the linear regression analysis.

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PSAC prevalence (see Table 6). We conducted the analysis with and without the studies from the Southern region which had the smallest sample sizes. Excluding these studies did not alter the results significantly, therefore we included all studies in the final analyses.

Table 6. Analysis of variance and coefficients—*S. mansoni, regression weighted by square root of PSACn*.

Source			**df**	**F-value**	**P- value**
Regression			88 (9)	25.63	<0.001
Prevalence in SAC			88 (1)	163.15	<0.001
African Regions (North, South, East, West and Central)			88 (4)	4.09	0.005
Treatment History			88 (2)	0.10	0.908
Prevalence in SAC * Treatment History			88 (2)	0.02	0.983
Coefficients	**Coef**	**SE Coef**	**95% CI Lower**	**95% CI Upper**	**P- value**
Constant	-0.137	0.065	-0.267	-0.008	0.038
Prevalence in SAC	0.7209	0.0564	0.6086	0.8333	<0.001
African Regions:					0.005*
North	0.000	0.000	0.000	0.000	
South	-0.198	0.191	-0.578	0.182	0.303
East	-0.037	0.057	-0.151	0.077	0.519
West	0.055	0.063	-0.071	0.181	0.385
Central	0.132	0.064	0.004	0.260	0.044
Treatment History:					0.908*
No previous treatment	0.000	0.000	0.000	0.000	
Treatment underway	-0.060	0.153	-0.364	0.244	0.696
Unknown	0.46	2.65	-4.81	5.73	0.861
SAC *Treatment History:					0.983*
No previous treatment	0.000	0.000	0.000	0.000	
Treatment underway	-0.026	0.200	-0.425	0.373	0.897
Unknown	-1.08	7.71	-16.43	14.28	0.889

Abbreviations: n–sample size, PSAC–preschool age children, SAC–School age children-square root arcsine transformed, df- degrees of freedom, Coef–coefficient, SE–Standard error.

*Overall P-value from regression model

Mathematical equation for the model: PSAC prevalence = A + bSAC prevalence + cAfrican Region +dTreatment History + eSAC*Treatment History

Where A is the constant coefficient and b,c,d and e are the variable coefficients.

https://doi.org/10.1371/journal.pntd.0008650.t006

Including the other variables and the interacting term in the model increased the *r*-squared value from 65.9% (95% CI = 54.7–77.1) to 74.5% (95% CI = 65.6–83.4) for *S. mansoni* and from 61.8% (95% CI = 46.4–77.2) to 70.3% (95% CI = 57.6–83.0) for *S. haematobium*. The correlation between PSAC and SAC prevalence was still significant. The results of this full model analysis are summarised in Tables 6 and 7. This analysis showed that the relationship between PSAC and SAC prevalences was not significantly altered by treatment history (*S. mansoni*, F value = 0.02, df = 88, 2, p = 0.983, and *S haematobium*, F value = 0.36, df = 53, 2, p = 0.966).

It is worth noting that although statistical significance was not detected for the effect of treatment history on PSAC infection prevalence or for the interaction factor between SAC infection prevalence and treatment history, for both *S. mansoni* and *S. haematobium*, SAC infection levels in populations where schistosome treatment was underway (e.g. through MDA), consistently mapped to lower PSAC infection levels (see Fig 5).

Discussion

This is the first study investigating an association between the prevalence of schistosomiasis in African school-aged children (SAC) and preschool-aged children (PSAC). This study is driven by the current need to define strategies to include PSAC in schistosomiasis treatment programmes. The current WHO guidelines for treating PSAC is to treat children on a case-by-

Table 7. Analysis of variance and coefficients—*S. haematobium, regression weighted by square root of PSACn*.

Source			**df**	**F-value**	**P- value**
Regression			53(11)	10.20	<0.0001
Prevalence in SAC			53(1)	57.42	<0.0001
African Regions (North, South, East, West and Central)			53(4)	1.21	0.319
Treatment History			53(2)	0.33	0.721
Diagnostic Technique			53(1)	0.01	0.910
Prevalence in SAC * Treatment History			53(2)	0.36	0.966
Coefficients	**Coef**	**SE Coef**	**95% CI Lower**	**95% CI Upper**	**P- value**
Constant	-0.095	0.102	-0.300	0.111	0.359
Prevalence in SAC	0.6776	0.894	0.497	0.8580	<0.0001
African Regions:					0.319*
North	0.00	0.00	0.00	0.00	
South	0.007	0.177	-0.350	0.364	0.968
East	0.1080	0.098	-0.090	0.306	0.276
West	0.019	0.102	-0.187	0.224	0.855
Central	0.181	0.181	-0.093	0.454	0.189
Treatment history:					0.721*
No previous treatment	0.00	0.00	0.00	0.00	
Treatment underway	-0.074	0.183	-0.433	0.295	0.688
Unknown	-0.483	0.706	-1.908	0.941	0.497
Diagnostic technique:					0.910*
Filtration	0.00	0.00	0.00	0.00	
Sedimentation	0.007	0.056	-0.106	0.119	0.614
SAC * Treatment history:					0.966*
No previous treatment	0.00	0.00	0.00	0.00	
Treatment underway	-0.107	0.210	-0.529	0.319	0.614
Unknown	0.544	0.789	-1.047	2.135	0.494

Abbreviations: n–sample size, PSAC–preschool age children, SAC–School age children-square root arcsine transformed, df- degrees of freedom, Coef–coefficient, SE–Standard error.

*Overall P-value from regression model

Mathematical equation for the model: PSAC prevalence = A + bSAC prevalence + cAfrican Region +dTreatment History + eDiagnostic Technique + fSAC*Treatment history

Where A is the constant coefficient and b,c,d,e and f are the variable coefficients.

https://doi.org/10.1371/journal.pntd.0008650.t007

case basis upon diagnosis, with the current formulation of PZQ [13]. As the global community prepares for the deployment of the paediatric formulation of PZQ (currently undergoing Phase III clinical trials), and for eventual elimination of schistosomiasis, one of the main operational questions that remain unanswered is how to quantify infection in PSAC, and to identify areas/populations to target for treatment. PSAC are out of the school system and therefore not as easily accessible as SAC. A further operational question is, at what level of schistosome endemicity would it be practical to conduct an infection survey in this age group to diagnose and treat infections? We investigated the relationship between currently available data on SAC and PSAC within the same community to determine if data from SAC, who are the current target of control programmes could be used to predict infection levels in PSAC.

Previous studies have investigated using SAC as a proxy group for community-wide prevalence levels, showing that SAC prevalence is closely related to adult prevalence, and therefore this age group could be used as a proxy group [24, 25]. Our findings confirm that this approach

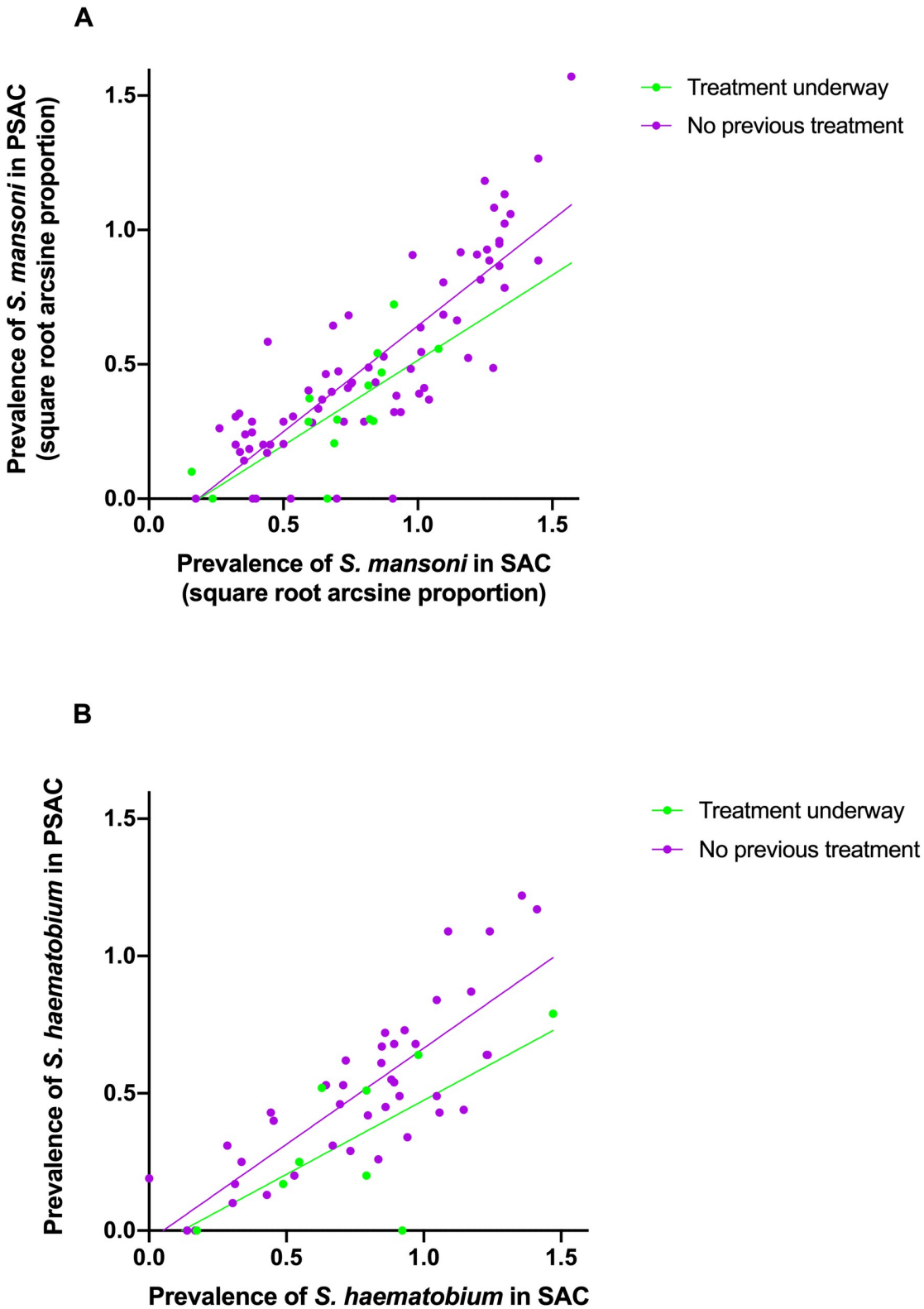

Fig 5. Scatterplots of PSAC vs SAC prevalence partitioned by, treatment history of the SAC for A) *S. mansoni* and B) *S. haematobium*.

https://doi.org/10.1371/journal.pntd.0008650.g005

can be extended to quantify infection levels in PSAC. Our results show that PSAC infection levels are significantly correlated with SAC prevalence levels, and indicate that it is possible to extrapolate PSAC prevalence levels from known SAC prevalence and treat them accordingly. This finding is not surprising as SAC tend to use the same water sources as the rest of the community, and their contact with infective water is related to activities of older family members [26], so that when a high proportion of SAC are infected, the community risk of infection accumulates rapidly.

Several countries have embarked on preventative chemotherapy through mass drug administration (MDA). Thus, some of the data we analysed came from communities undergoing MDA programmes targeted at SAC, or in a few cases, targeting both SAC and adults, while others had not experienced MDA in the past 50 years; no current MDA programme includes PSAC. Therefore, we included the SAC, or SAC and adult's treatment history in our analyses. The current study indicated that the relationship between PSAC and SAC prevalences did not change significantly depending on treatment history of the study population. This finding may be due to the small sample sizes in the study because, albeit not significant in areas undergoing treatment, SAC levels translated to lower PSAC levels than in areas which had not experienced any treatment. In other words, to get the same levels of PSAC prevalence in areas that had undergone treatment, higher SAC prevalence levels were required than when compared to that in areas without treatment, for both *S. mansoni* and *S. haematobium*. In areas undergoing MDA, treatment of SAC has the potential of lowering levels of contamination of shared water sources with the infection, especially if treatment coverage is high and includes adults in the community; in this case, PSAC are exposed to lower levels of infection. In two examples where treatment was underway, one for S. *mansoni* and one for *S. haematobium*, infection prevalence in PSAC was greater than in SAC, presumably because the PSAC are excluded from the MDA programmes.

The maintenance of a relationship between PSAC and SAC prevalences during treatment, is relevant for most countries targeting SAC via preventative chemotherapy, and will need to include PSAC. This will become particularly prominent if the paediatric formulation of PZQ currently undergoing phase III clinical trials becomes available. This means that even data from national treatment impact assessment exercises that are mostly conducted by surveying SAC, are informative for developing strategies for treating PSAC. Thus, PSAC prevalence can be inferred from current levels of SAC prevalence data (as can be found in the Espen WHO database [27]), which can now be used to map prevalence levels of schistosomiasis in PSAC in Africa. Our results suggest that where prevalence of schistosomiasis infection is high in SAC, limited surveys could be used to validate infection in PSAC to justify preventive chemotherapy. This may obviate the need to conduct extensive surveys for schistosomiasis in PSAC. This is also in keeping with the WHO recommendation that given the financial and human resources required for surveys, existing epidemiological data including historical records should be used to generate baseline information for implementing a control strategy [6].

Unsurprisingly the relationship between SAC and PSAC indicates that infection levels in PSAC are zero or undetectable below a certain prevalence of SAC infection. This is due to both the low sensitivity of the widely used parasitological schistosomiasis diagnostic tests in detecting low intensity infections [28], as well as the infection transmission dynamics. Infection accumulates with age, with most children being exposed to infection within their first year of life, mainly as a result of increasing water contact and exposure patterns [29, 30]. While we can determine exposure to infection in the very young children serologically, infection detection by egg counts is less reliable because of the low levels of infection the children carry [29]. There is also the operational challenge of collecting stool and urine samples from the younger PSAC, who may not be toilet-trained; in this study PSAC age ranged from <1 year up to 6

years. Similarly, the number of samples collected would also affect the accuracy of diagnosis and not all studies indicated the number of urine or stool samples collected for the parasitology diagnosis.

Where we had sufficient data to investigate the influence of parasite egg collection methods, we found that for example with *S. haematobium*, the choice of egg collection methods, did not affect the PSAC infection prevalence. This is not surprising as both methods relied on enumerating parasite eggs excreted in urine, and thus suffer the same limitations of low sensitivity in people with low infection intensities [30].

Looking at methodological aspects, as with most meta-analyses and literature reviews, some publication bias can occur. Thus, we conducted a quality assessment exercise of the data published in the source reference. We then conducted the analyses both including and excluding the data from studies scoring low on the quality assessment, and as this did not alter the results, the data from all studies satisfying the inclusion criteria were kept in the analysis.

There were other possible analyses that would have been more informative, but sample sizes were too small for the data to be partitioned or analysed by those categories. For example, for both *S. mansoni* and *S. haematobium*, only one study reported previous, but no current MDA in the SAC. These both had their most recent MDA 2 years prior to the study quantifying SAC and PSAC infection, where the one examining *S. haematobium* used Niridazole instead of PZQ. The impact of MDA on the relationship between PSAC and SAC infection levels after cessation of MDA in SAC thus remains unexplored, but may likely not vary significantly due to infection rebound [31]. It would also have been informative to have the data for both SAC and PSAC in each age group, but this more granular data were not presented in the source publication. We attempted to capture some of the heterogeneity in the data by including factors such as the African sub-region where the data were collected, but other factors which could not be determined from the information presented in the publications might also account for the variation in the PSAC infection prevalence (the model explained 74.5% of the variation in *S. mansoni* and 70.3% in *S. haematobium*) This includes factors such as number of parasitology samples collected, number of slides prepared for diagnosis and age-related water contact. Nonetheless, the strong correlation between the two variables (i.e. PSAC and SAC prevalence) in these heterogeneous studies suggest that when quantifying infection in the same community with less heterogeneity, the SAC infection prevalence should be a more informative proxy for PSAC infection prevalence.

We determined the proportion of study populations classified into a lower infection level i.e. misclassified into a lower WHO classifications category based on the SAC levels. For *S. mansoni*, 4/89 (4.5%) of PSAC prevalences were underestimated and therefore misclassified, based on the interpolation of the regression equation. For *S. haematobium*, 4/54 (7.4%) of PSAC prevalences were misclassified. There are several reasons that could explain this, an example is treatment history. One of the *S. mansoni* studies reported that MDA was underway in SAC, and had similar prevalences in SAC and PSAC. The published source studies reported treatments via MDA but there was no indication if there had been any individual treatment provisions through health facilities. For *S. haematobium*, one study reported higher infection prevalence in PSAC than SAC, and the population had not had MDA targeted at the SAC. However, no explanation was given for this observation. The fact that even with the heterogeneities highlighted, over 90% of the studies were classified into the correct WHO classifications category based on the SAC levels from the regression model indicates that the SAC prevalences can be extended as a proxy for infection levels in PSAC as they are already being used for the adult population.

In conclusion, it has already been demonstrated that in schistosome endemic areas, PSAC are at high risk of schistosome infection and disease and therefore should be included in

treatment strategies. Here, we have demonstrated that schistosomiasis infection levels in PSAC are highly correlated to levels in SAC, giving the potential for using already existing data from SAC as a predictor of infection levels in PSAC, for both *S. mansoni* and *S. haematobium*. Critically, we have shown that the relationship between PSAC and SAC infection levels is informative during ongoing community or SAC-targeted treatment, as well as in communities that have not received schistosome treatment. With the increasing concern of the health impact of schistosomiasis in this PSAC, using already existing SAC data to inform PSAC chemotherapy could accelerate and facilitate the treatment of PSAC who are in urgent need of treatment. This will allow endemic countries to move closer towards the goal of eliminating schistosomiasis as a public health burden by 2025 [32].

Supporting information

S1 Checklist. PRISMA checklist.
(DOCX)

S1 Table. Participant and methodology characteristics of studies included in meta-analysis containing *S. mansoni* data.
(DOCX)

S2 Table. Participant and methodology characteristics of studies included in meta-analysis containing *S. haematobium* data.
(DOCX)

S3 Table. Quality appraisal of articles containing prevalence data of *S. mansoni* infection in preschool and school age children in Africa.
(DOCX)

S4 Table. Quality appraisal of articles containing prevalence data of *Schistosoma haematobium* infection in preschool and school age children in Africa.
(DOCX)

Author Contributions

Conceptualization: Rivka M. Lim, Takafira Mduluza, Lester Chitsulo, Francisca Mutapi.

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Writing – original draft: Rivka M. Lim, Francisca Mutapi.

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